Genetic testing for germline variants in homologous recombination repair genes, other than *BRCA1* and *BRCA2*, in patients with suspected hereditary cancer syndromes

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Introduction

Homologous recombination repair (HRR) is the cellular mechanism for error-free repair of DNA double-strand breaks. Pathogenic germline variants in *BRCA1* and *BRCA2* lead to HRR deficiency associated with breast, ovarian, prostate, pancreatic cancers and are sensitive to PARP inhibitors (PARPi). Defects in HRR genes beyond *BRCA1/2* could also result in HRR deficiency and sensitize the tumor to PARPi, thus expanding the subset of patients that can benefit from these targeted therapy cancer drugs.

Objective

The main objective of this study was to assess the variant spectrum of relevant genes involved in the HRR pathway, excluding *BRCA1* and *BRCA2*.

Methods

Sample: 56 genomic DNAs from patients with personal and family history of cancer.

Testing of genes involved in HRR: NGS (TruSight® Hereditary Cancer and Trusight Cancer gene panels, using MiSeq and Next Seq 500 from Illumina) of *ATM, BAP1, BLM, BRIP1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, NBN, PALB2, RAD51C* and *RAD51D* genes.

Sequence alignment and variant annotation: DRAGEN Enrichment and Variant Interpreter - Illumina®, VEP, HSF, Alamut, VarSome and several databases (ex. HGMD, gnomAD, dbSNP).

Variant classification : according to ACMG-AMP[1]; variants of uncertain significance (VUS) were further classified with the stepwise *ABC* system[2] to distinguish VUS based in insufficient knowledge from those based in conflicting evidence.

Confirmation of pathogenic/likely pathogenic variants: Sanger sequencing or MLPA.

Results

We identified 156 SNVs and 1 CNV, of these 132 were classified as benign/likely benign. Seven clinically actionable variants were found in 10,7% of the patients (Fig.1 and Table 1):

- **4 pathogenic variants:** 3 in *FANCA*, *FANCD2* and *FANCI* (Fig. D, E, F) give rise to premature stop codons and 1 CNV in *FANCA* (deletion of exons 38 and 39 (Fig. G);
- **3 likely pathogenic variants:** 2 in *BLM* and *FANCI* (Fig. A, B) affecting splicing and 1 frameshift in *FANCG* (Fig. C).

Classification of 18 VUS (ACMG) with the ABC system resulted in:

- 8 class 0 VUS (normal finding)
- 7 class E VUS (potential interest)
- 3 class D VUS (low penetrance).

In addition, 7 SNVs were classified as hypomorphic alleles according to functional studies available from the literature.

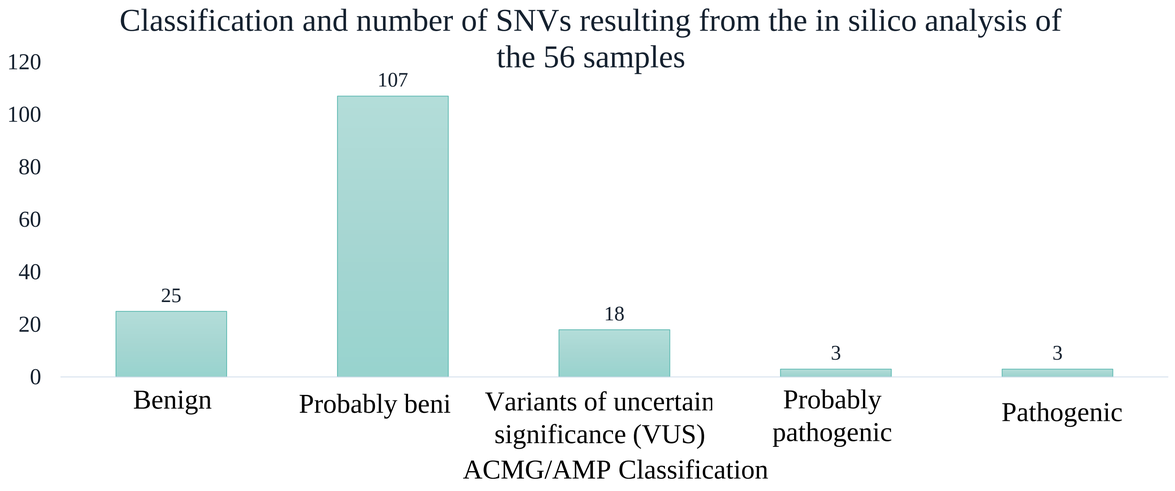
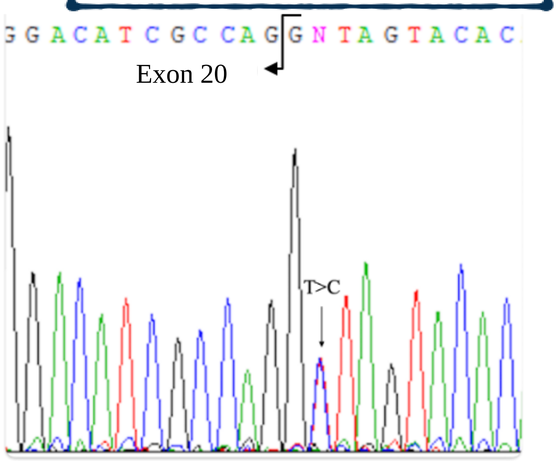
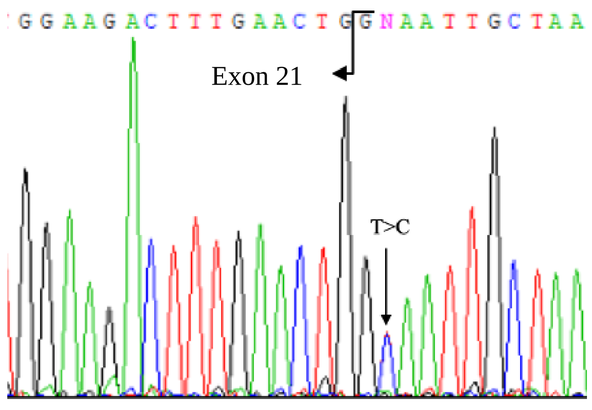


Figure 1: Classification and number of SNVs resulting from the in silico analysis of the 56 samples

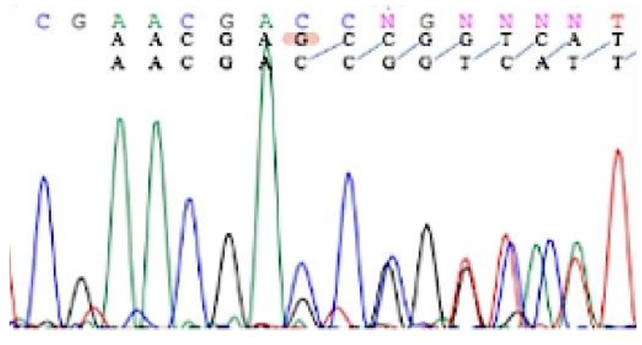
A. *BLM*:c.3874+2T>C p.(?)



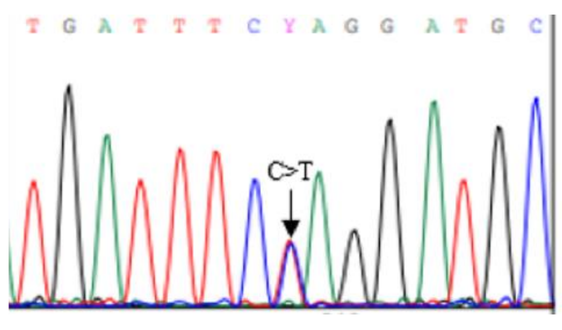
B. *FANCI*:c.2169+2T>C p.(?)



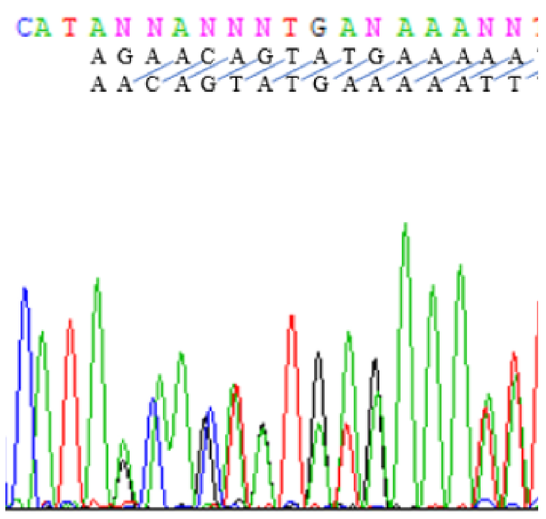
C. *FANCG*:c.67del p.(Leu23SerfsTer13)



D. *FANCD2*:c.1981C>T p.(Gln661Ter)



E. *FANCI*:c.3772_3773del p.(Glu1258ThrfsTer3)



F. *FANCA*:1709_1715+9del p(?)

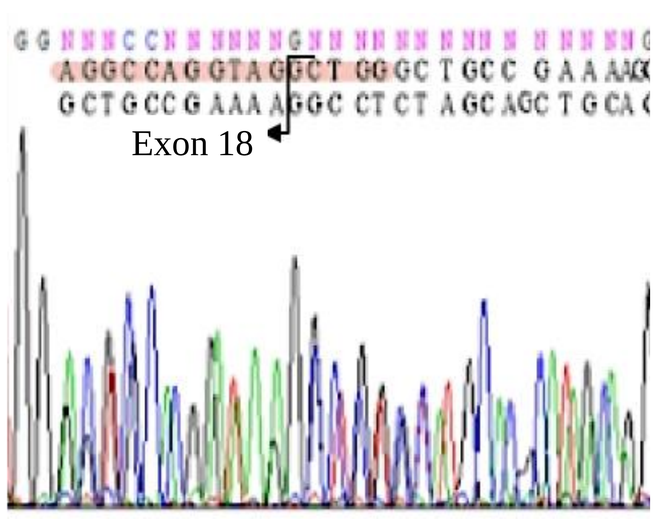
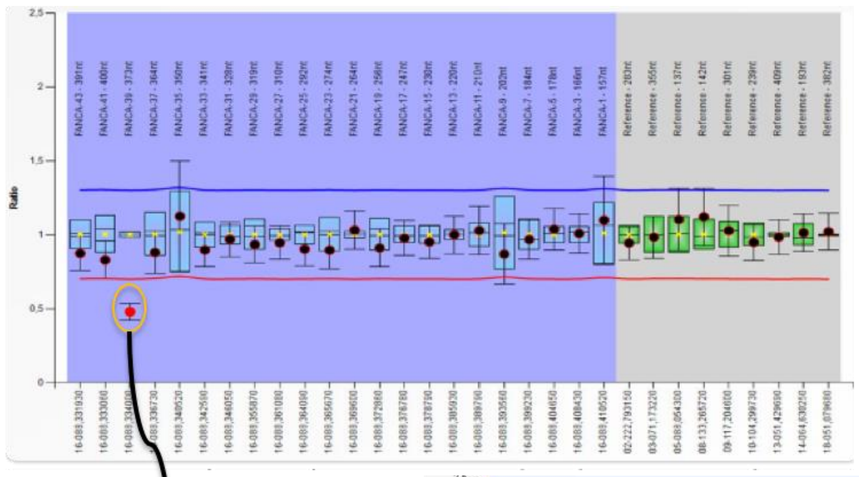


Table 1- Variants classified as likely pathogenic/pathogenic

Gene	cDNA	Protein	ACMG/AMP classification	Exon/ Intron	Sample ID
BLM	c.3874+2T>C	p.?	Probably pathogenic	20	HRR-47
FANCA	c.1709_1715+9del	p.?	Pathogenic	18/18	HRR-13
FANCD2	c.1981C>T	p.(Gln661Ter)	Pathogenic	22	HRR-56
FANCG	c.67del	p.(Leu23SerfsTer13)	Probably pathogenic	1	HRR-55
FANCI	c.2169+2T>C	p.?	Probably pathogenic	21	HRR-33
	c.3772_3773del	p.Glu1258ThrfsTer3	Pathogenic	36	HRR-48



G. *MLPA results - FANCA gene of sample HRR-47*

Ratio:
≥1,3 – loss
≤0,7 - gain

Exon 39
Ratio – 0,48

Exon 38
Ratio – 0,51

Discussion

According to the literature, in hereditary breast cancer patients, the *FANCI* gene is altered in 3.06%, *FANCA* in 2.1%, *BLM* in 1.56%, *FANCD2* in 2.89% and *FANCG* in 1.43%.[3] We obtained similar results: 3.57% for FANCI and *FANCA* and 1.78% for *BLM*, *FANCG*, *FANCD2*. The presence of pathogenic alterations in these genes can be an inclusion criterion for subsequent clinical studies.

Conclusions

This study allowed us to evidence:

i) The importance of extending the molecular study beyond *BRCA1/2* to other genes involved in HRR;

ii) Some variants require functional and family studies in order to elucidate their biological impact, improve genotype/phenotype correlations and ultimately to establish their pathogenicity;

iii) The HRR genes tested in this work could potentially be considered for specific and clinical studies involving PARPi therapy.

1 - de S Richards et al 2015 (doi:10.1038/gim.2015.30); 2 - de G Houge et al 2022 (doi:10.1038/s41431-021-00903-z); 3 - https://www.mycancergenome.org/content/biomarkers/