P38

BRCA1 and *BRCA2* variants identified in patients with a personal/familial history of Hereditary Breast/Ovarian Cancers and other Hereditary Cancer Syndromes: challenges related with variants of uncertain significance

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INTRODUCTION

Screening for *BRCA1* and *BRCA2* variants in patients with Hereditary Breast/Ovarian Cancer (HBOC) or other Hereditary Cancer Syndromes (HCS) is performed using next-generation sequencing (NGS), allowing the detection of a high number and types of variants.

The growing use of PARP inhibitors (PARPi) in the treatment of patients with homologous recombination-deficient tumors contributes to an increasing number of patients being screened for *BRCA1* and *BRCA2* variants even when family history of HBOC/HCS is absent. These approaches result in a higher number of identified variants that need to be classified.

The goals of this study, apart from identifying pathogenic and likely pathogenic variants, were to discuss variants of uncertain significance (VUS) their uncertainties and impact on patients and family members.

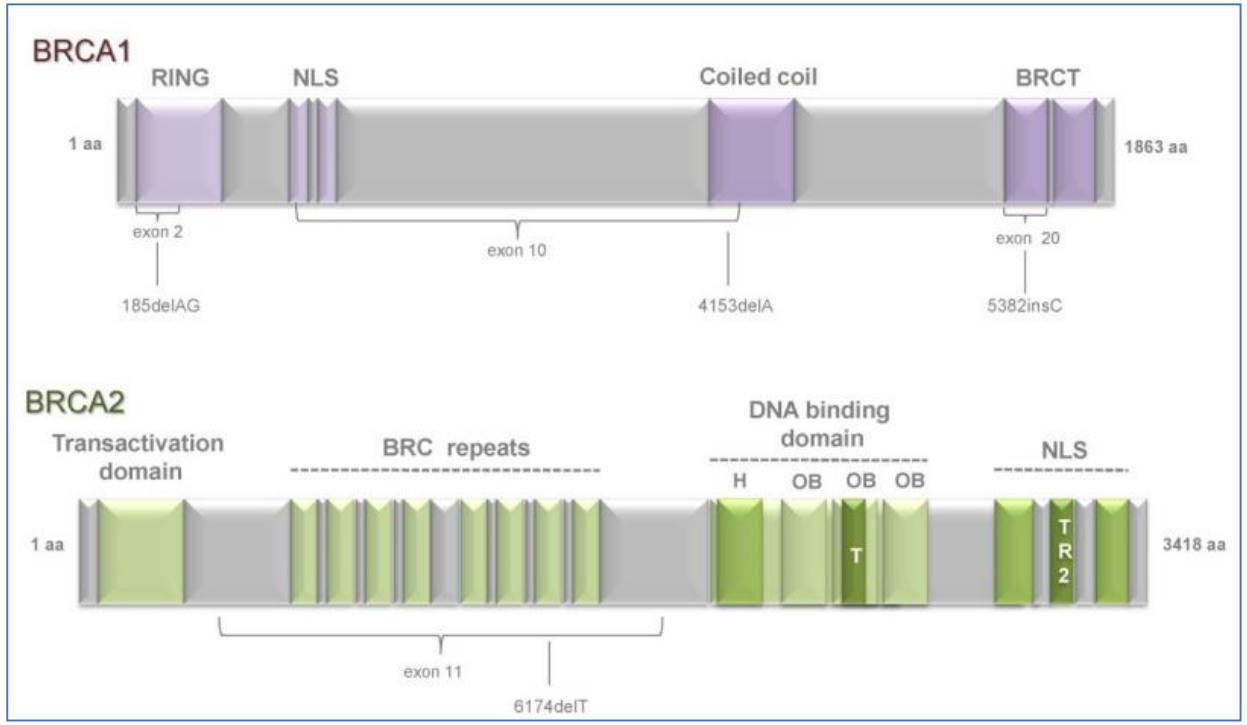


Fig.1 - Schematic representation of functional domains within BRCA1 and BRCA2 proteins and the position of several founder mutations. *BRCA1* is composed of 23 exons and *BRCA2* 27 exons[1].

MATERIALS AND METHODS

BRCA1 and *BRCA2* were analyzed in 207 patients mainly with HBOC/HCS. The Portuguese founder mutation *BRCA2*:c.156_157ins*Alu* was screened by allele specific PCR before the NGS assay which was performed using TruSight® Cancer (TSC) and MiSeq.

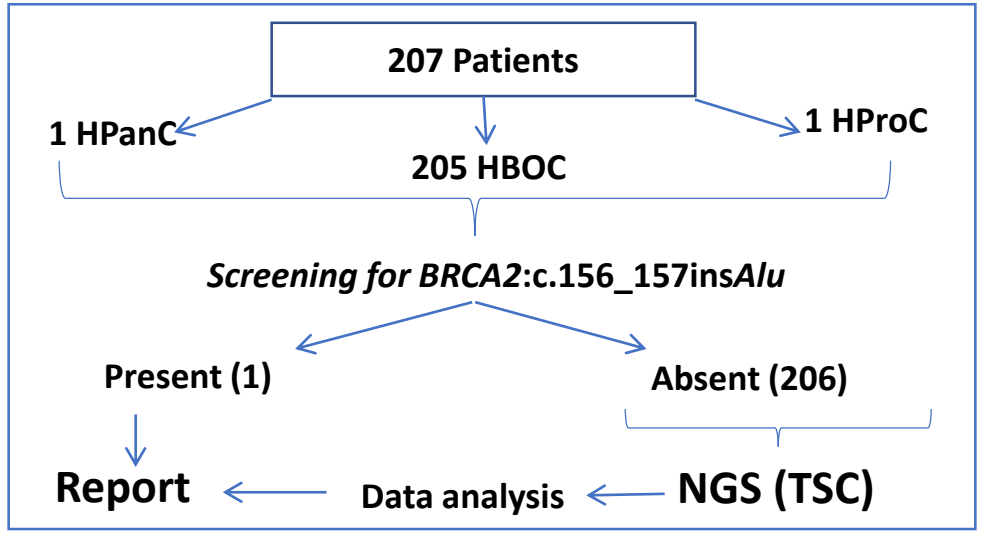


Fig.2 - Simplified samples workflow. HBOC - Hereditary Breast/Ovarian Cancer, HPanC - Hereditary Pancreatic Cancer, HProC - Hereditary Prostate Cancer. TSC - TruSight® Cancer

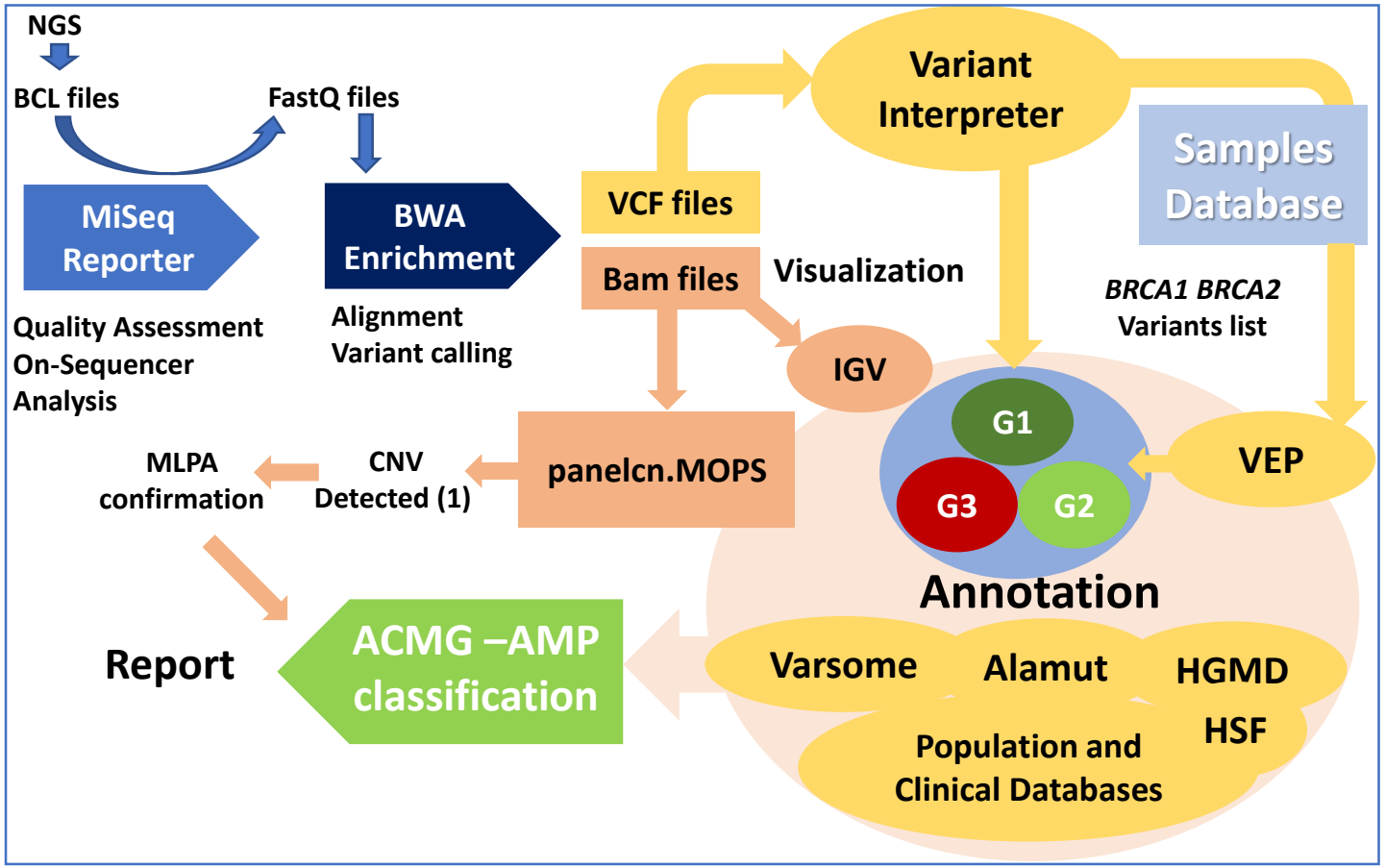


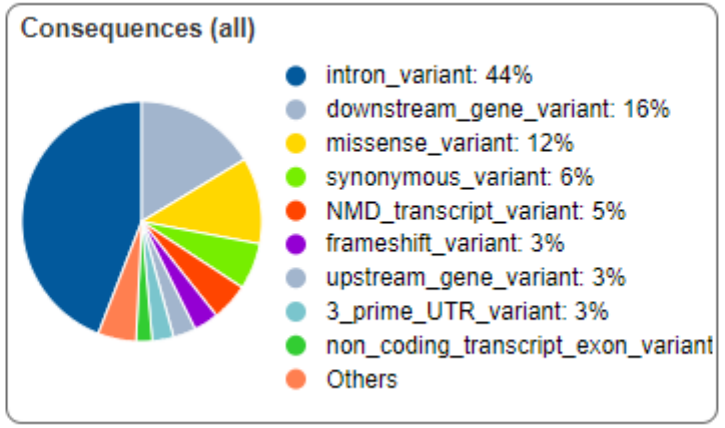
Fig.3 - Simplified bioinformatic pipeline used for analysis of NGS data. After the creation of a *BRCA1* and *BRCA2* unique list of variants, annotation was performed using several softwares, clinical and population databases. Variants were divided in 3 groups (G1, G2 and G3) according to allele frequency.

Annotation was performed with Variant Interpreter, Variant Effect Preditor (VEP), Human Splice Finder (HSF), Alamut, Varsome, and Integrative Genomic Viewer (IGV). Variants were divided in 3 groups according to allele frequency in population and classified according to ACMG-AMP guidelines[2]. Group 1 - allele frequency (AF) higher than 5%, Group 2 - AF lower than 5% and equal/higher than 1% and Group 3 - AF lower than 1%.

RESULTS

In *BRCA1* and *BRCA2*, 45 and 96 variants were detected respectively. *BRCA1* Group 1 presented **10** variants, Group 2 - **4** variants and Group 3 - **31** variants. In this last Group, according to ACMG-AMP classification, we detected **6 pathogenic** (P) variants and **9 VUS**.

Category	Count
Variants processed	44
Variants filtered out	0
Novel / existing variants	5 (11.4) / 39 (88.6)
Overlapped genes	3
Overlapped transcripts	35
Overlapped regulatory features	3



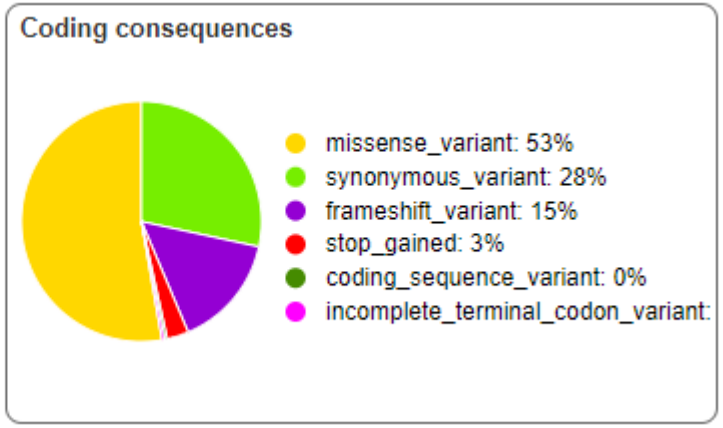
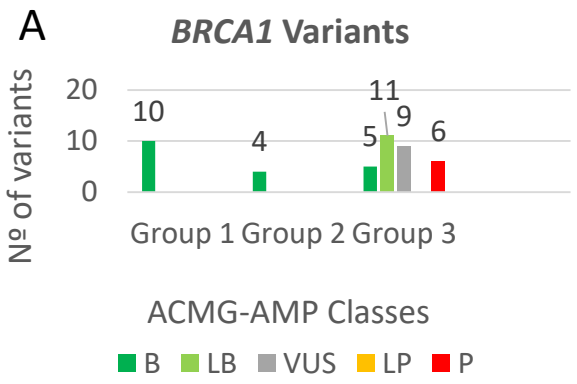
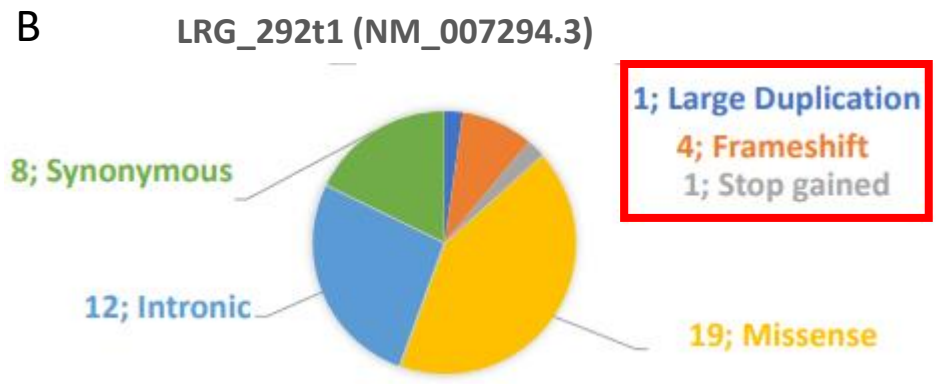


Fig.4 - *BRCA1* variants annotated with VEP. 45 Variants were submitted to VEP, including a duplication of exon 12, but *BRCA1*:c.-19-8T>C was not processed. This variant was annotated later using rs number (rs 398122626).

A



B



C

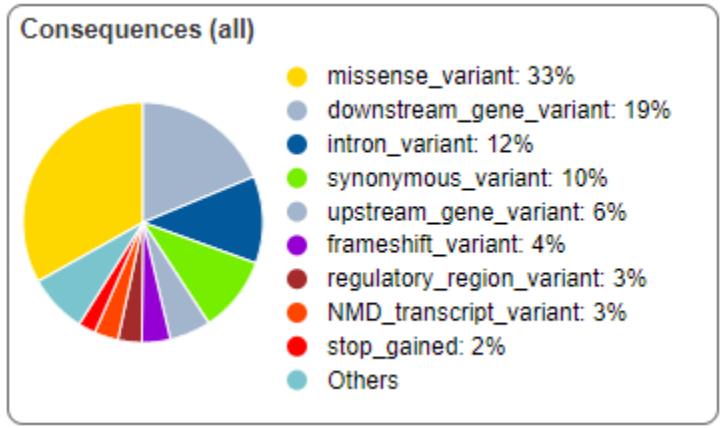
HGVSc (LRG_292t1)	HGVSp (LRG_292p1)	Exon Nº
c. (4185+1_4186-1)_(4357+1_4358-1)dup	p.?	12
c.2036delinsCC	p.(Lys679Thrfs*4)	10
c.3189delC	p.(Ser1064Valfs*9)	10
c.470_471delCT	p.(Ser157*)	7
c.5266dupC	p.(Gln1756Profs*74)	19
c.1945G>T	p.(Glu649*)	10

Fig.5 - *BRCA1* variants types and numbers. (A) Variants group distribution according to ACMG-AMP classification (B- Benign, LB - Likely Benign, VUS - Variants of Uncertain Significance, LP- Likely Pathogenic, P- Pathogenic). (B) Number of variants identified in the main transcript NM_007294.3. (C) *BRCA1* pathogenic variants.

RESULTS

BRCA2 Group 1 presented **14** variants, Group 2 - **5** variants and in Group 3 we found **77** variants (**11 P**, **3 likely pathogenic** (LP), and **22 VUS**). We highlight that in Group 3, VUS were more frequent than P and LP variants.

Category	Count
Variants processed	96
Variants filtered out	0
Novel / existing variants	11 (11.5) / 85 (88.5)
Overlapped genes	4
Overlapped transcripts	16
Overlapped regulatory features	5



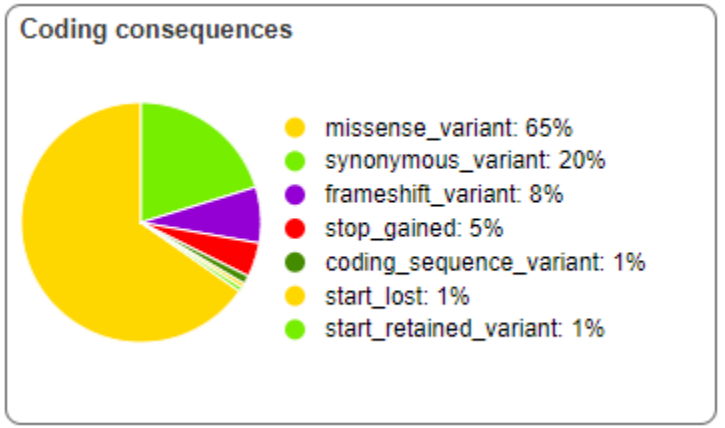
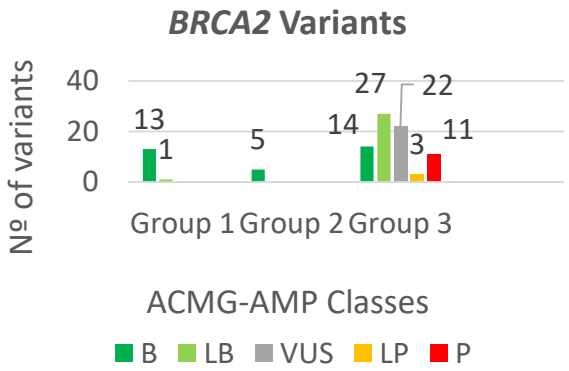
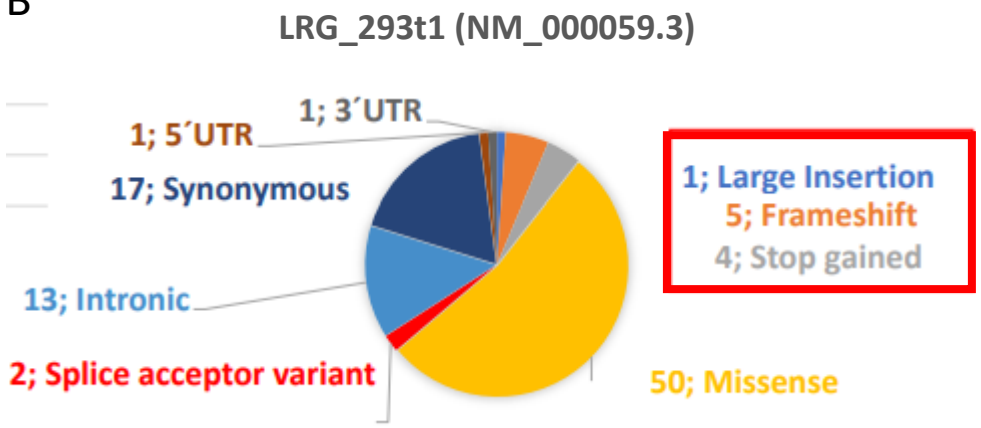


Fig.6 - *BRCA2* variants annotated with VEP. 96 Variants were submitted to VEP, including the *BRCA2*:c.156_157ins*Alu*.

A



B



C

HGVSc (LRG_293t1)	HGVSp (LRG_293p1)	Exon Nº
c.156_157ins*Alu*	p.?	3
c.1308_1309delGA	p.Lys437ArgfsTer14	10
c.370dela	p.Met124TrpfsTer12	4
c.5722_5723delCT	p.Leu1908ArgfsTer2	11
c.6001dupT	p.Ser2001PhefsTer2	11
c.478delG	p.Val160TyrfsTer12	6
c.10222A>T	**p.Lys3408Ter**	**27**
c.7588C>T	p.Gln2530Ter	15
c.7738C>T	p.Gln2580Ter	16
c.9976A>T	p.Lys3326Ter	27

Fig.7 - *BRCA2* variants types and numbers. (A) Variants group distribution according to ACMG-AMP classification (B- Benign, LB - Likely Benign, VUS - Variants of Uncertain Significance, LP- Likely Pathogenic, P- Pathogenic). (B) Number of variants identified in the main transcript NM_000059.3 (C) *BRCA2* - 9 P variants and 1 LB stop gained variant (c.10222A>T).

RESULTS and DISCUSSION

A

HGVSc (LRG_292t1)	HGVSp (LRG_292p1)	Exon Nº
c.1796A>G	p.Asn599Ser	10
c.2351C>T	p.Ser784Leu	10
c.3221G>T	p.Arg1074Ile	10

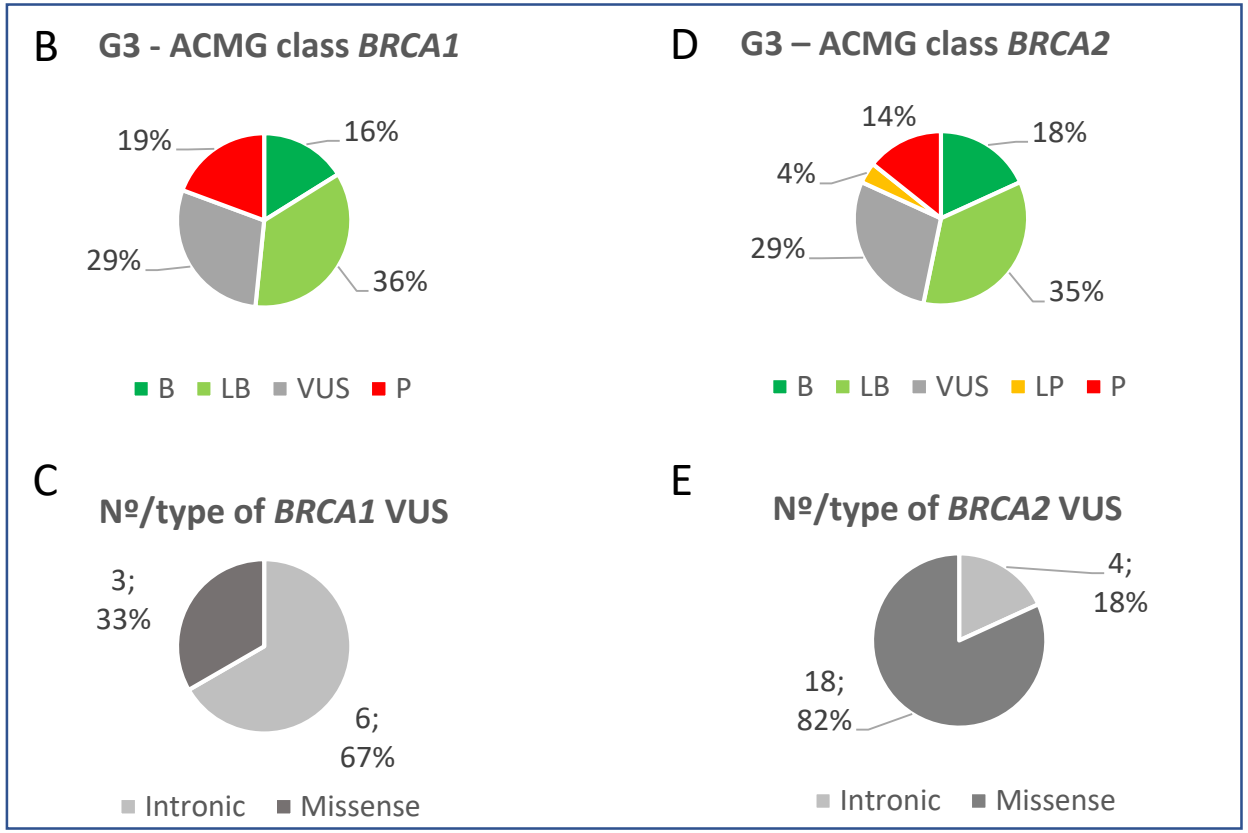


Fig.8 - *BRCA1* and *BRCA2 VUS* variants types and numbers. (A) *BRCA1* missense variants. (B) G3 *BRCA1* ACMG classes % and (C) types of VUS variants. (D) *BRCA2* missense variants. (E) G3 *BRCA2* ACMG classes % and (F) types of VUS variants. Variants in bold are not reported in gnomAD exomes and gnomAD genomes.

F

HGVSc (LRG_293t1)	HGVSp (LRG_293p1)	Exon Nº
c.280C>T	p.Pro94Ser	3
c.676A>C	p.Thr226Pro	8
c.1096T>G	p.Leu366Val	10
c.1499G>A	p.Gly500Asp	10
c.1714G>A	p.Val572Ile	10
c.1741T>C	p.Ser581Pro	10
c.3485C>G	p.Ala1162Gly	11
c.3755C>A	p.Ser1252Tyr	11
c.3814A>G	p.Met1272Val	11
c.4123G>A	p.Glu1375Lys	11
c.4900T>C	p.Phe1634Leu	11
c.4915G>A	p.Val1639Ile	11
c.5228G>A	p.Ser1743Asn	11
c.7166G>A	p.Arg2389Lys	14
c.8482A>G	p.Ile2828Val	19
c.8518A>G	p.Ile2840Val	20
c.8902A>G	p.Thr2968Ala	22
c.9616C>A	p.Gln3206Lys	26

DISCUSSION

Among Group 3, 29% of *BRCA1* and *BRCA2* variants were classified as VUS, considering ACMG-AMP guidelines. The majority of variants classified as VUS was based in the absence or extremely lower allele frequency in population databases, although the majority of *in silico* analysis results suggested that these variants might be tolerant/non pathogenic. VUS missense variants were located at exon 10 in *BRCA1* and mainly at exon 10 and 11 in *BRCA2*. VUS give rise to difficulties related to management of patients and families. Functional studies of missense or putatively affecting splicing VUS are of major importance to assess their pathophysiologic impact, as some of them may be hypomorphic and reclassified as P/LP. After additional studies, some VUS may have impact in therapeutic decisions (e.g. PARPi) as well as in patient's cancer-risk management protocols, including appropriate genetic counselling and screening them in selected family members. We predict that new challenges related to VUS will emerge.

References **1.** Gorodetska I. *et al* (2019); 10(9): 2109-2127. doi: 10.7150/jca.30410. **2**. Richards S. *et al* (2015); 17 (5):405-423. 5. doi:10.1038/gim.2015.30. (**Acknowledgements** FCT/MCTES, Toxicogenomics and Human Health(UIDB/00009/2020).GenomePT project (POCI-01-0145-FEDER-022184). **All authors declare no conflicts of interest.**