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EPIDEMIOLOGY

BRCA1 and *BRCA2* germline mutation analysis from a cohort of 1267 patients at high risk for breast cancer in Brazil

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Abstract

We determined the frequency and mutational spectrum of *BRCA1* and *BRCA2* in a series of patients at high risk for developing breast cancer from Brazil. A total of 1267 patients were referred for *BRCA* genetic testing, and no obligation of fulfilling criteria of mutation probability methods for molecular screening was applied. Germline deleterious mutations in *BRCA1/2* (i.e., pathogenic/likely pathogenic variants) were identified in 156 out of 1267 patients (12%). We confirm recurrent mutations in *BRCA1/2*, but we also report three novel mutations in *BRCA2,* not previously reported in any public databases or other studies. Variants of unknown significance (VUS) represent only 2% in this dataset and most of them were detected in *BRCA2*. The overall mutation prevalence in *BRCA1/2* was higher in patients diagnosed with cancer at age > 35 years old, and with family history of cancer. The present data expand our knowledge of *BRCA1/2* germline mutational spectrum, and it is a valuable clinical resource for genetic counseling and cancer management programs in the country.

Keywords *BRCA1* · *BRCA2* · Germline mutation · Genetic testing · Hereditary breast and ovarian cancer · Brazilian population

Introduction

BRCA1 and *BRCA2* are two major breast cancer susceptibility genes in the context of large, multiple-case families, segregating both early onset breast cancer and ovarian cancer [1–4]. Pathogenic variants in these genes account for 20%–40% of the familial breast cancer cases [5]. Female carriers of *BRCA1/2* mutations have an increased lifetime risk of developing both breast and ovarian cancers [6–8]. Currently, germline genetic screening for *BRCA1/2* mutations has been routinely applied for high-risk patients, being an essential tool for cancer prediction and clinical management. The *BRCA1/2* mutation carriers can benefit from intensive surveillance, prophylactic surgery, and chemoprevention, reducing their risk of developing breast cancer [9–11]. Additionally, clinical trials of targeted drugs, such as poly ADP-ribose polymerase inhibitors, shed light on the treatment promise for advanced breast cancer patients who carry *BRCA1/2* mutations [12, 13]. Integration of genetic counseling and testing is then paramount for diagnosis of hereditary breast and ovarian cancer syndrome in mainstream oncology.

Since the identification of *BRCA* gene, extensive efforts have been dedicated to its sequence data analysis. However, the clinical interpretation of variants in *BRCA1/2* sometimes can be challenging. To date, more than 20,000 unique variants, including missense, nonsense, frameshift, and splicing variants as well as large rearrangements have been described in both genes (www.brcaexchange.org). While some of them can be confidently predicted to be pathogenic since they affect the structure and function of the gene, a significant proportion of them are rare missense with unknown functional consequences. Moreover, the prevalence and spectrum of *BRCA1/2* mutations is extremely variable among certain populations and ethnic groups [14–16]. Several studies have been conducted to evaluate the epidemiologic characteristics of *BRCA1/2*

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mutations in diverse populations, however, most of them focused on white populations from Europe and North America, Asian, and African American populations. In particular, the Brazilian population has one of the most heterogeneous genetic constitutions in the world with a predominant tri-hybrid composition (Native Americans, Europeans, and Africans) in an extensive admixture [17]. Although the number of studies increased in the past few years [18, 19], the mutational spectrum of *BRCA1/2* in the Brazilian population remains largely unknown. Usually, the sample size of the studies is typically small, and little is known about the prevalence of in *BRCA1/2* germline mutations in Brazilian patients. Hence, there is a need for better understanding the germline mutational landscape of these high-penetrance genes and cancer risk prediction, so that appropriate genetic counseling and clinical management programs could be implemented. In this study, we present a comprehensive analysis of *BRCA1/2* germline mutations from a cohort of 1,267 patients at high risk for developing breast cancer examined in a diagnostic routine.

Patients and methods

Casuistic

This is a retrospective, observational study that compiled clinical and molecular data of *BRCA* genetic testing results from patients investigated routinely in a private laboratory from Brazil (Diagnostics of America S.A.—DASA), between January 2017 and March 2019. A total of 1267 consecutive, unrelated individuals were referred for molecular screening either because of personal or family history of breast and/or ovarian cancer. The genetic testing was performed upon a medical request, which is linked to the patient risk stratification for hereditary breast and ovarian cancer. The clinicians followed international guidelines of a selected panel of experts who define criteria for testing individuals at high risk for hereditary breast and ovarian cancer [20, 21]. Briefly, the genetic testing was offered to patients who met at least one of the following criteria:

(i) Individual from a family with a known deleterious *BRCA1* and *BRCA2* mutation;
(ii) Personal history of breast cancer and ≥ 1 of these: (a) diagnosed at age ≤ 45 years; (b) diagnosed at age ≥ 50 years with ≥ 1 close blood relatives with breast cancer at age 50 years and/or ≥ 1 close blood relatives with epithelial ovarian cancer at any age; (c) two breast primaries when first breast cancer diagnosis occurred at age ≤ 50 years; (d) diagnosed at age ≤ 60 years with a triple negative breast cancer; (e) diagnosed at age ≤ 50 years with a limited family history; (f) diagnosed at any age, with ≥ 2 close blood relatives with breast and/or epithelial ovarian cancer at any age; (g) diagnosed at any age with ≥ 2 close blood relatives with pancreatic cancer at any age; (h) close male blood relative with breast cancer; (i) individual of ethnicity associated with higher mutation frequency (e.g., Ashkenazi Jewish), (j) personal history of epithelial ovarian cancer, male breast cancer, or pancreatic cancer at any age with ≥ 2 close blood relatives with breast and/or ovarian cancer and/or pancreatic cancer at any age;
(iii) No personal history of breast cancer, but with a family history including ≥ 1 of these: (a) first- or second-degree blood relative meeting any of the above criteria; (b) third-degree blood relative with breast cancer and/or ovarian cancer with ≥ 2 close blood relatives with breast cancer (≥ 1 with breast cancer at age ≤ 50 years) and/or ovarian cancer.

Clinical information was collected from the genetic test requisition form, which was filled by the patients, and included: sex, age at cancer diagnosis or referral for genetic testing, history of unilateral or bilateral breast cancer, triple negative breast cancer status, personal history of other types of cancer, and family history of cancer.

This study was approved by the Ethics Committee from Hospital 9 de Julho (CAAE: 53,253,821.8.0000.5455), and all patients provided an informed consent for genetic testing.

BRCA genetic testing and variants analysis

Genomic DNA samples were extracted from peripheral blood cells following standard procedures. All patients were subjected to a comprehensive *BRCA* testing (full *BRCA* sequencing and multiplex ligation-dependent probe amplification—MLPA). The genetic testing was performed using different methodologies, including full gene analysis by Sanger or Next Generation Sequencing (NGS), and MLPA for analysis of large genomic rearrangements (LGR). In particular, the NGS-based capture method used was either the Ion AmpliSeq BRCA1/2 Panel (ThermoFischer, USA) or the Hereditary Cancer Panel designed by SOPHiA GENETICS (Switzerland), that encompasses the entire coding sequences, and each intron/exon boundaries of *BRCA1/2* genes. Sequencing data were analyzed using the SOPHiA DDM™ software with a specific algorithm for variant calling and annotation, which also include LGR detection. LGR were confirmed by MLPA (SALSA MLPA P002-D1 and P045-B3—MRC Holland); the amplified products were electrophoretically separated using the ABIPrism310 genetic analyzer and interpreted with the Coffalyser analysis software (MRC Holland).

The Human Genome Variation Society (HGVS) nomenclature guidelines (http://varnomen.hgvs.org/) was used for variants annotation, and the ClinVar database (www.ncbi.nlm.nih.gov/clinvar/) was used to determine the biological significance of all reported variants. The detected variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines [22] as Pathogenic (P), Likely Pathogenic (LP), Benign (B), Likely Benign (LB), or Variants of Unknown Significance (VUS). The common variants (B/LB), i.e., those frequently reported in curated databases were disregarded from this study. Sanger sequencing was performed to validate all clinically relevant variants (P, LP, and VUS). For novel variants, Breast Cancer Information Core (http://research.nhgri.nih.gov/bic), BRCA Share (formerly known as UMD, http://www.umd.be/), LOVD (http://www.lovd.nl/3.0/home), ARUP (http://arup.utah.edu/database/ BRCA/) and BRCA Exchange (http://brcaexchange.org/) databases were also checked. As an additional data source for variants classification, we also consulted the Mastermind database (https://mastermind.genomenon.com/), and reports in the literature from the Brazilian population and worldwide studies [19, 23]. Importantly, for supporting evidence of pathogenicity of novel variants and VUS, we used the Alamut Visual Plus™ software (SOPHiA GENETICS, Switzerland) that assesses both the probability of protein sequence damage, and de novo creation of splice sites, based on NNSplice and MaxEnt algorithms. Further, to estimate the impact of novel variants and VUS on protein structure we also specify three evidence categories (population frequency data, variant type and location, and case-level data) as recommended by Harrison 2019 [24].

Results

Out of the 1267 patients referred for molecular screening, 1080 had a negative result, and the remaining 187 individuals were either positive for a clinically relevant variant (P/LP) or VUS in the *BRCA* gene; the corresponding frequency of P/LP variants and VUS in our cohort were 12% and 2%, respectively (Supplementary Fig. 1A). The Supplementary Table 1 shows that, as expected, the largest proportion of patients investigated were in fact females (98%). Among the individuals with P/LP variants, 93 (60%) of them carried a mutation on *BRCA1*, and 63 (40%) carried a mutation on *BRCA2*. Regarding the frequency of VUS, no significant difference was observed between both genes (Supplementary Fig. 1B). The distribution of the different types of mutations on *BRCA1/2* is shown in Supplementary Fig. 1C; nonetheless, frameshift mutations are the most frequent deleterious variant in both genes, accounting for nearly half of all cases. Of note, three distinct and novel presumably disease-causing variants were detected in *BRCA2* (Supplementary Fig. 1D). The spectrum of all *BRCA1*/2 mutations identified in our cohort is presented in Tables 1 and 2.

The most frequent P/LP variants on *BRCA1/2*, here defined as a mutation found in three or more individuals, are unequally distributed in the five different regions of Brazil (Fig. 1). The *BRCA1* c.5266dup (p. Gln1756ProfsTer74) variant, highlighted in bold, was the most common in our cohort, detected in 20 individuals and in all geographical areas of the country. In particular, the novel variants, each of them representing a single case, were observed in the Midwest, Southeastern, and South region of Brazil. However, the number of patients referred for *BRCA* genetic testing is not equal in the country (Supplementary Table 2). The North region has the lowest number of individuals investigated in this dataset, even though the proportion of negative cases, P/LP variants and VUS was very similar among the five regions (Supplementary Table 3).

Overall, the mean age of all patients investigated was 42 years old (Fig. 2A). The mutation prevalence in patients with cancer, by age group, was particularly high on women aged between 36 and 40 years old (Fig. 2B); late-onset cancer (≥ 45 years old) indeed represents most cases, as shown on Fig. 2C. Also, the mutation prevalence was significantly higher in patients who also had a family history of cancer (Fig. 2D). Supplementary Table 4 presents a more detailed description of mutation prevalence in patients with or without diagnosis of breast and/or ovarian cancer, according to family history and the corresponding frequency of P/LP, VUS and negative results in our cohort. Unilateral breast cancer represents the largest number of cases in our cohort (67% and 81% in patients with P/LP variants and VUS, respectively). Besides, patients with P/LP variants have approximately 3 times more bilateral breast cancer (18%) when compared to those with VUS (6%) (Supplementary Tables 5, 6 and 7). Notably, patients with P/LP variants who have a first-degree family history with a diagnosis of ovarian cancer add up to more than double the number of patients with VUS.

Discussion

In the present study, we evaluated the frequency and mutational spectrum of *BRCA1/2* in a series of patients being at high risk for hereditary breast and ovarian cancer. No obligation of fulfilling criteria of mutation probability methods for molecular screening was applied. In our cohort, 12% (156/1267) of the patients carried a deleterious germline *BRCA* mutation (93 *BRCA1* and 63 *BRCA2*), being the vast majority very rare, found in only one or

Table 1 Spectrum of all *BRCA1* germline mutations detected in this study

BRCA1—NM_007294.4			
P/LP variants			
Variation	Amino acid change	Coding impact	rs number
Variants detected once ($n=32$; 33,3%)			
c.441 + 2 T > A	p.(?)	Splicing	rs397509173
c.116G > A	p.(Cys39Tyr)	Missense	rs80357498
c.4675 + 1G > A	p.(?)	Splicing	rs80358044
c.131_132del	p.(Cys44Ter)	Nonsense	rs1597911705
c.3916_3917del	p.(Leu1306AspfsTer23)	Frameshift	rs80357678
c.1961del	p.(Lys654SerfsTer47)	Frameshift	rs80357522
c.4480G > T	p.(Glu1494Ter)	Nonsense	rs80357148
c.131G > T	p.(Cys44Phe)	Missense	rs80357446
c.181 T > G	p.(Cys61Gly)	Missense	rs28897672
c.2405_2406del	p.(Val802GlufsTer7)	Frameshift	rs80357706
c.178C > T	p.(Gln60Ter)	Nonsense	rs80357471
c.2727_2730del	p.(Asn909LysfsTer90)	Frameshift	rs80357605
c.4357 + 1G > C	p.(?)	Splicing	rs80358027
c.5057A > G	p.(His1686Arg)	Missense	rs730882166
c.4414del	p.(Leu1472PhefsTer33)	Frameshift	rs1567779686
c.1380dup	p.(Phe461IlefsTer19)	Frameshift	rs80357714
c.1795_1799del	p.(Asn599SerfsTer12)	Frameshift	rs886039968
c.1016dup	p.(Lys339ArgfsTer2)	Frameshift	rs80357569
c.470_471del	p.(Ser157Ter)	Nonsense	rs80357887
c.798_799del	p.(Ser267LysfsTer19)	Frameshift	rs80357724
c.188 T > A	p.(Leu63Ter)	Nonsense	rs80357086
c.3598C > T	p.(Gln1200Ter)	Nonsense	rs62625307
c.303 T > A	p.(Tyr101Ter)	Nonsense	rs80356936
c.5251C > T	p.(Arg1751Ter)	Nonsense	rs80357123
c.5463_5464insT	p.(His1822SerfsTer8)	Frameshift	rs1057518636
c.1123_1124delCTinsA	p.(Leu375LysfsTer19)	Frameshift	rs2053989334
c.547 + 2 T > A	p.(?)	Splicing	rs80358047
c.68_69del	p.(Glu23ValfsTer17)	Frameshift	rs80357914
c.3362del	p.(Asn1121IlefsTer8)	Frameshift	rs80357865
c.1016dup	p.(Val340GlyfsTer6)	Frameshift	rs80357569
c.34C > T	p.(Gln12Ter)	Nonsense	rs80357134
c.5074 + 1del	p.(?)	Splicing	rs1597825560
Variants detected twice ($n=3$; 3,1%)			
c.4834C > T	p.(Gln1612Ter)	Nonsense	rs786202064
c.4484G > T	p.(Arg1495Met)	Missense	rs80357389
c.3817C > T	p.(Gln1273Ter)	Nonsense	rs80357208
Variants detected three or more times ($n=7$; 7,3%)			
c.5266dup	p.(Gln1756ProfsTer74)	Frameshift	rs80357906
c.3331_3334del	p.(Gln1111AsnfsTer5)	Frameshift	rs80357701
c.5074 + 2 T > C	p.(?)	Splicing	rs80358089
c.211A > G	p.(Arg71Gly)	Missense	rs80357382
c.190 T > C	p.(Cys64Arg)	Missense	rs80357064
c.3756_3759del	p.(Ser1253ArgfsTer10)	Frameshift	rs80357868
c.1687C > T	p.(Gln563Ter)	Nonsense	rs80356898
VUS			
Variants detected once ($n=14$; 100%)			
c.1985A > G	p.(His662Arg)	Missense	rs80357494

Table 1 (continued)

BRCA1—NM_007294.4			
P/LP variants			
Variation	Amino acid change	Coding impact	rs number
c.2885A > G	p.(Glu962Gly)	Missense	rs780367532
c.3657G > C	p.(Glu1219Asp)	Missense	rs80356876
c.4893 T > A	p.(Ser1631Arg)	Missense	rs80356850
c.4934G > C	p.(Arg1645Thr)	Missense	rs70953661
c.1601A > G	p.(Gln534Arg)	Missense	rs80357173
c.3406C > A	p.(Pro1136Thr)	Missense	rs431825395
c.*291C > T	p.(?)	UTR 3'	rs878854928
c.332A > C	p.(Glu111Ala)	Missense	rs80357312
c.835C > T	p.(His279Tyr)	Missense	rs1380919500
c.3975G > T	p.(Arg1325Ser)	Missense	rs?
c.2215A > G	p.(Lys739Glu)	Missense	rs56329598
c.1459G > T	p.(Val487Phe)	Missense	rs369588942
c.5102 T > C	p.(Leu1701Pro)	Missense	rs1597820325

Frequency of *BRCA1* variants detected three or more times: c.5266dup (21.5%); c.3331_3334del (15.1%); c.5074 + 2 T > C and c.211A > G (6.5%); c.190 T > C, c.3756_3759del, and c.1687C > T (3.2%)

Reported mutations in *BRCA1*, showing all Pathogenic/Likely Pathogenic (P/LP) variants and Variants of Unknown Significance (VUS) identified in our cohort

two individuals. Particularly, the most frequent *BRCA1* mutation, accounting for 21.5% of all *BRCA1* mutations, was the c.5266dup (p.Gln1756ProfsTer74), while the *BRCA2* c.2808_2811del (p.Ala938ProfsTer21), accounting for 12.7%, was the most frequent of all *BRCA2* mutations.

The BRCA1 c.5266dup (p.Gln1756ProfsTer74) was found in 20 individuals and among all geographical regions in Brazil. This mutation, also known as 5382insC or 5385insC, is a known founder mutation in individuals of Ashkenazi Jewish ancestry [25], and present at appreciable frequency in several European countries [26]. Noteworthy, 19 individuals have European descent between those with the BRCA1 c.5266dup mutation. The median age at breast cancer diagnosis in this series was 35 years old, and four individuals were discriminated as mutation carriers without breast, ovarian or any other type of cancer. Three out four carriers had a family history of breast or ovarian cancer in at least one first- and second-degree relatives at young age. Unilateral triple negative breast cancer was seen in 6/20 and three patients presented bilateral breast cancer.

The *BRCA2* c.2808_2811del (p.Ala938ProfsTer21) was found in 8 individuals from the Southern and Southeastern regions of Brazil. Five women with unilateral breast cancer harboring this specific mutation were detected in this series. The median age at breast cancer diagnosis in these cases was 35 years old, which include one male carrier at the age of 40, with family history of hereditary breast and ovarian cancer syndrome, and two females' carriers at the age of 23 and 61. The 23 years old female has a family history of breast cancer that included three cases of first- and second-degree relatives. An additional high burden of other types of cancers has been observed in the families with *BRCA2* c.2808_2811del carriers, in particular, an enrichment of prostate cancer. It is relevant to mention that among all *BRCA2* mutations a few were seen in several populations in different ethnic groups, but the c.2808_2811del is in the top 10 most frequent mutations detected between all ethnicities [27]. Recently, the *BRCA2* c.2808_2811del was described as the second most common *BRCA2* variant in a Brazilian populational study [19].

Notably, considering the most frequent P/LP variants in this dataset (Fig. 1), the three most common are in accordance with a recent worldwide *BRCA1/2* mutational spectrum report, which listed *BRCA1* c.5266dup, *BRCA1* c.3331_3334del and *BRCA2* c.2808_2811del, among the top 5 *BRCA1/2* mutations in Brazil [27]. With particular interest, we highlight a *BRCA2* mutation in our cohort that are strongly associated with the African American population. The c.6405_6409del (p.Asn2135LysfsTer3) was the second most common *BRCA2* mutation, and it was present in six individuals from the Southern and Southeastern regions of Brazil. Three women with unilateral breast cancer, two triple negative and a 60 years old patient with ovarian cancer harbor this pathogenic variant. Within this group there was also a female carrier at the age of 22 with family history of hereditary breast cancer. Of note, the c.6405_6409del was found among the 10 most common mutations in Brazil, African American and South/Central America [27]. It is worth mentioning that the geographical distribution of *BRCA1/2* mutations in Brazil, as shown in

Table 2 Spectrum of all *BRCA2* germline mutations detected in this study.

BRCA2—NM_000059.4			
P/LP variants			
Variation	Amino acid change	Coding impact	rs number
Variants detected once ($n=31$; 49,2%)			
c.6656C > G	p.(Ser2219Ter)	Nonsense	rs80358893
c.8682del	**p.(Val2895PhefsTer14)**	**Frameshift**	**rs?**
c.7987del	p.(Glu2663LysfsTer10)	Frameshift	rs886040738
c.4380_4381del	p.(Ser1461LeufsTer4)	Frameshift	rs397507715
c.6585dup	p.(Lys2196Ter)	Missense	rs886040669
c.2 T > G	p.(Met1?)	Start-loss	rs80358547
c.4030_4035delinsC	p.(Asn1344HisfsTer6)	Frameshift	rs886040509
c.3601_3602delinsT	**p.(Asn1201SerfsTer8)**	**Frameshift**	**rs?**
c.8363G > A	p.(Trp2788Ser)	Nonsense	rs80359080
c.4936_4939del	p.Glu1646GlnfsTer23	Frameshift	rs863224465
c.1670 T > G	p.Leu557Ter	Nonsense	rs80358452
c.517-1G > A	p.(?)	Splicing	rs81002849
c.6596del	p.(Thr2199IlefsTer7)	Frameshift	rs876658294
c.5200dup	p.(Glu1734GlyfsTer9)	Frameshift	rs1555284103
c.7480C > T	p.(Arg2494Ter)	Nonsense	rs80358972
c.4005dup	p.(Phe1336IlefsTer2)	Frameshift	rs397507701
c.3659dup	**p.(Tyr1220Ter)**	**Nonsense**	**rs?**
c.7900del	p.(Met2634TrpfsTer14)	Frameshift	rs1566244864
Deletion exons 15–16	p.(?)	Rearrangement	rs–
c.8009C > T	p.(Ser2670Leu)	Missense	rs80359035
c.3744_3747del	p.(Ser1248ArgfsTer10)	Frameshift	rs80359403
c.8488-1G > A	p.(?)	Splicing	rs397507404
c.93G > A	p.(Trp31Ter)	Nonsense	rs80359214
c.9117G > A	p.(Pro3039=)	Synonymous	rs28897756
c.156_157insAlu	p.(?)	Alu Insertion	rs–
c.4329del	p.(Phe1443LeufsTer5)	Frameshift	rs?
c.6407 T > G	p.(Leu2136Ter)	Nonsense	rs?
c.1296_1297del	p.(Asn433GlnfsTer18)	Frameshift	rs80359276
c.6275_6276del	p.(Leu2092ProfsTer7)	Frameshift	rs11571658
c.6024dup	p.(Gln2009AlafsTer9)	Frameshift	rs80359554
c.8351G > A	p.(Arg2784Gln)	Missense	rs80359076
Variants detected twice ($n=6$; 9,5%)			
c.5471dup	p.(Asn1824LysfsTer5)	Frameshift	rs80359515
c.7738C > T	p.(Gln2580Ter)	Nonsense	rs80358999
c.9382C > T	p.(Arg3128Ter)	Nonsense	rs80359212
c.4740_4741dup	p.(Glu1581ValfsTer37)	Frameshift	rs864622401
c.4808del	p.(Asn1603ThrfsTer14)	Frameshift	rs397507743
c.1138del	p.(Ser380ValfsTer19)	Frameshift	rs80359264
Variants detected three or more times ($n=4$; 6,5%)			
c.2808_2811del	p.(Ala938ProfsTer21)	Frameshift	rs80359351
c.6405_6409del	p.(Asn2135LysfsTer3)	Frameshift	rs80359584
c.9371A > T	p.(Asn3124Ile)	Missense	rs28897759
c.5616_5620del	p.(Lys1872AsnfsTer2)	Frameshift	rs80359525
VUS			
Variants detected once ($n=12$; 70,5%)			
c.1564G > C	p.(Gly522Arg)	Missense	rs80358442
c.5096A > G	p.(Asp1699Gly)	Missense	rs80358732

Table 2 (continued)

BRCA2—NM_000059.4			
P/LP variants			
Variation	Amino acid change	Coding impact	rs number
c.5729A > G	p.(Asn1910Ile)	Missense	rs276174863
c.6024G > C	p.(Lys2008Asn)	Missense	rs56324666
c.6095C > T	p.(Ala2032Val)	Missense	rs786202701
c.619A > G	p.(Thr207Ala)	Missense	rs80358858
c.7457A > G	p.(Asn2486Ser)	Missense	rs786203755
c.811G > A	p.(Gly271Arg)	Missense	rs786204274
c.8755G > T	p.Gly2919Cys	Missense	rs1454684155
c.9945del	p.(Glu3316AsnfsTer2)	Frameshift	rs431825381
c.3045G > T	p.(Lys1015Asn)	Missense	rs?
c.67 + 25 T > C	p.(?)	Intronic	rs1226106794
Variant detected twice ($n = 1$; 5,9%)			
c.3032C > G	p.(Thr1011Arg)	Missense	rs80358548
Variant detected three times ($n = 1$; 5,9%)			
c.280C > T	p.(Pro94Ser)	Missense	rs80358531

Frequency of *BRCA2* variants detected three or more times: c.2808_2811del (12.7%); c.6405_6409del (9.5%); c.9371A > T and c.5616_5620del (4.8%)

Reported mutations in *BRCA2*, showing all Pathogenic/Likely Pathogenic (P/LP) variants and Variants of Unknown Significance (VUS) identified in our cohort. Mutations highlighted in bold are novel, not described in any public databases

Fig. 1 and Supplementary Tables 2 and 3, highlights the unequal frequency of patients referred to genetic testing and access of supplementary health in the country. The Brazilian Society of Medical Genetics (https://www.sbgm.org.br/) points out that one third of the country`s geneticists are located in the state of São Paulo, which certainly influences molecular investigation and treatment of the patients.

Nearly all P/LP in *BRCA1/2* described here were previously reported, except for three novel variants in *BRCA2*: (i) a frame shift mutation starting at codon Asn1201 [c.3601_3602delinsT; p.(Asn1201SerfsTer8)] detected in a 64 years old woman with breast cancer, and family history for breast and prostate cancer; (ii) a 1 bp duplication in exon 11 that interrupts the reading frame prematurely at position 1220[c.3659dup; p. (Tyr1220Ter)], detected in a 60 years old woman, African descent with bilateral triple negative breast cancer, and no family history for breast, ovarian or other cancer type; and (iii) a 1 bp deletion in exon 21 leading to a frameshift [c.8682del; p.(Val2895PhefsTer14)] effect in a 28 years old Italian descendant woman with bilateral triple negative breast cancer and familial history of Hereditary breast, ovarian and colon cancer.

Regarding the frequency of VUS, this class of variant represent only 2% (31/1267) in our cohort. VUS impose a challenge relating to the management and surveillance of carriers as well as in risk assessment. To categorize these variants, six different in silico tools were applied (i.e., phyloP; Grantham dist; Align GVGD; SIFT and Mutation Taster), and their classification was also based on consulted databases and complementary sources as previously described. A total of 31 unique VUS were identified tin the patients (14 and 17 in *BRCA1* and *BRCA2*, respectively), and none of them was detected neither at carriers of *BRCA1/2* P/LP variants nor present more than once. Out of the 14 *BRCA1* VUS, 13 are reported on ClinVar database to have unknown clinical significance, and only one variant has no records on public databases (*BRCA1* c.3975G > T) (Table 1). Interestingly, we identified one non-coding VUS in *BRCA1* localized in the 3´UTR region, the c.*291C > T, which was detected in a 57 years old woman with unilateral breast cancer and family history of breast cancer. A functional luciferase assay previously showed that the c.*291C > T increased *BRCA1* 3'UTR activity [28]. Although there is an increasing number of data associating germline non-coding variants with higher cancer risk, a co-segregation analysis was not possible to be made because the VUS was reported only in this woman. It is noteworthy that this non-coding variant was only described in breast cancer cases [29]. Among the 17 *BRCA2* VUS, 14 are reported on ClinVar database to have unknown clinical significance, while 3 of them have no records on public databases (BRCA2 c.67 + 25 T > C, c.3045G > T and c.8755G > T) (Table 2). In particular, the BRCA2 c.8755G > T variant was previously described in a Brazilian series where it also was provisionally classified as VUS [18]; in silico tools predicts that this variant exerts a possible effect nearest the splice

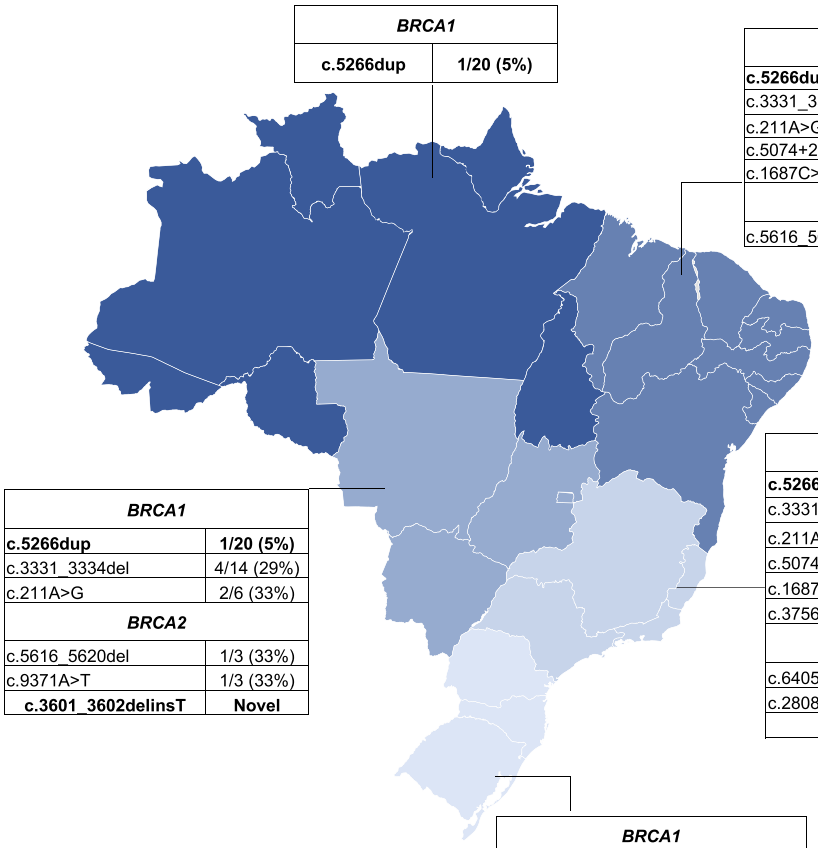

Fig. 1 Geographical distribution of the most frequent *BRCA1/2* mutations in Brazil. A total of eleven mutations are displayed in the map, and these were defined as most frequent because they were found in three or more individuals in the present cohort. The *BRCA1* c.5266dup (p. Gln1756ProfsTer74) variant, highlighted in bold, was the most common, detected in 20 individuals and in all geographical areas of the country

site. Considering that there is a degree of inconsistency between these in silico predictions tools, it is impossible to draw any conclusions on the pathogenicity of VUS. Nonetheless, an integrated strategy which will include co-segregation analysis, tumor pathology data, as well as functional assays is needed to complete a comprehensive assessment of pathogenicity of these variants.

The characteristics of *BRCA1/2* mutation carriers in our cohort (i.e., the overall mutation prevalence was higher in patients diagnosed with cancer > 35 years old, and with a family history of cancer) were similar to other studies. Although it was expected that patients with a *BRCA1/2* mutation were more likely to have bilateral breast cancer, in the current study unilateral breast cancer represent the largest number of cases. Nonetheless, patients with P/LP variants in *BRCA1/2* have approximately 3 times more bilateral breast cancer when compared to those with VUS. Different definitions of familial breast cancer and genetic testing methods for *BRCA1/2* mutations between the studies may influence the results. Currently, the National Comprehensive Cancer Network (NCCN) guidelines are the most widely used criteria for testing and inform insurance coverage decisions. Even though the criteria have expanded over time to be more inclusive, recent data suggest that expansion of testing may be appropriate [30, 31]. The mutation detection rate in patients with

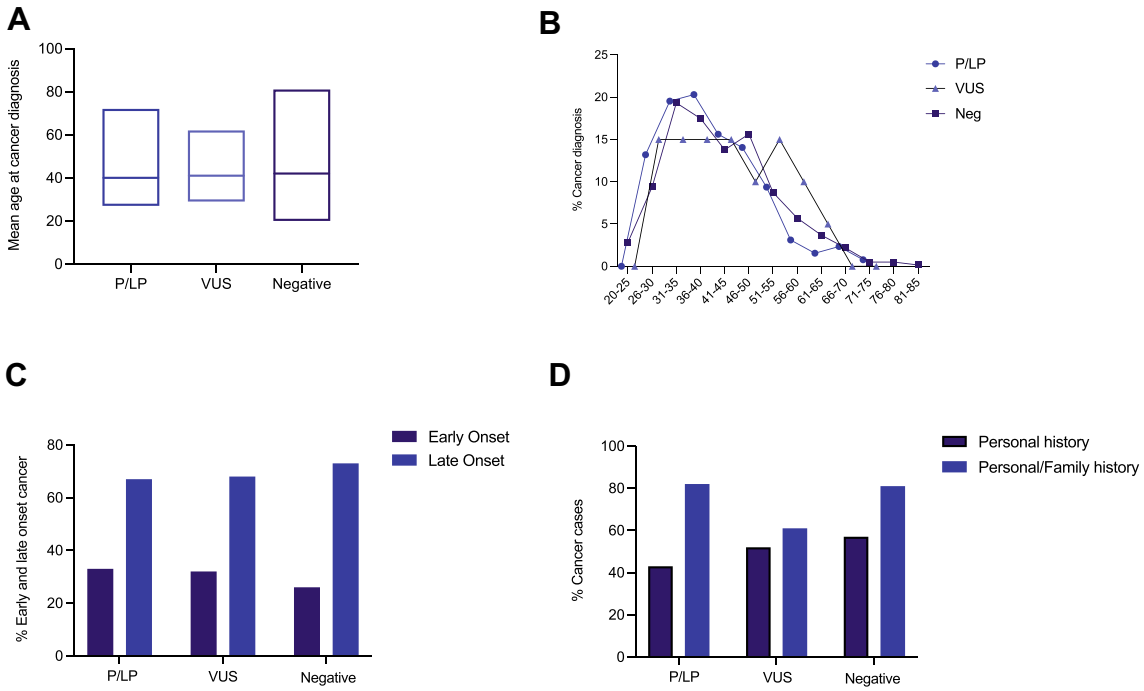

Fig. 2 Characteristics of *BRCA1/2* mutation carriers in this study. **A** The mean age at cancer diagnosis of all patients investigated was 42 years old. **B** Mutation prevalence in patients with cancer by age group. **C** Frequency of patients with early (≤ 35 years old) and late-onset cancer (≥ 45 years old). **D** Frequency of patients with personal history of cancer at diagnosis and with family history of cancer

breast cancer tested based on NCCN guidelines varied widely depending on the type and number of criteria met. In general, *BRCA* detection rates were significantly increased when the reason for testing was age ≤ 45 at time of diagnosis or having a known family history of a *BRCA* mutation, but specific clinical scenarios such as triple negative breast cancer status also have been associated with a high risk for *BRCA* mutations. We followed the NCCN guidelines for genetic testing referral in our patient cohort, which may explain the higher diagnostic yield (12%) when compared to the estimated frequency of 5–10% of all hereditary breast and ovarian cases attributed to P/LP germline mutations in *BRCA1* and *BRCA2* [32].

In summary, the present data expand our knowledge of the frequency and *BRCA1/2* germline mutational spectrum in Brazil, being a valuable clinical resource for genetic counseling and cancer management programs in the country. Also, based on the increase interest for *BRCA* genetic testing among individuals who are at high risk of carrying a mutation, our data may help provide guidelines in the future for patients with breast cancer who should undergo molecular screening.

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Data availability All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the ethics committee from Hospital 9 de Julho (CAAE: 53253821.8.0000.5455), and all patients provided an informed consent for genetic testing.

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