Detection of Inherited Mutations in Brazilian Breast Cancer Patients Using Multi-Gene panel Testing

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Abstract

Genetic diversity of germline variants in breast cancer (BC) predisposition genes, is unexplored in miscegenated people, such as Latin American populations. We evaluated 1,662 Brazilian BC patients, who underwent hereditary multi-gene panel testing (20–38 cancer susceptibility genes), to determine the spectrum and prevalence of (likely) pathogenic variants (P/LP) and variants of uncertain significance (VUS). In total, 161 (9.7%) participants carried germline P/LP variants in BRCA1/2 and 162 (9.7%) in other cancer predisposition genes. Overall, 341 distinctive P/LP variants were identified in 22 genes, including BRCA1(28%), BRCA2(19%), TP53(11%), MUTYH heterozygous (10%), ATM(9%), CHEK2(6%), and PALB2(5%). The Brazilian variant TP53 R337H (c.1010G > A, p.Arg337His), detected in 1.6% of BC patients and 0.09% of reference controls (RC), was strongly associated with odds of disease (OR = 17.67; 95%CI:9.21–34.76; p < 0.001). Heterozygous MUTYH c.1187G > A and MUTYH c.536A > G, detected in 0.78% (0.90% RC) and 0.48% (0.40% RC) of the patients, respectively, were not associated with the odds of BC, the former with OR = 0.87 (95%CI:0.49–1.53; p = 0.63) and the latter with OR = 1.20 (95%CI:0.58–2.49; p = 0.63). Besides, 766 individuals (46.1%) had 1 or more VUS. Concluding, the use of multi-gene panel testing doubled the identification of mutation carriers in Brazilian BC patients. Special attention should be given to TP53 mutations.

1. Introduction

Breast cancer (BC) is the most common cancer in women worldwide. In Brazil, an average of 66,280 women are diagnosed with carcinoma of the breast every year, accounting for 29.7% of all cancers in the female population[1]. Inherited pathogenic variants in highly penetrant predisposition genes are thought to be involved in about 10% of BC cases. Among the hereditary forms, the most frequent events are germline pathogenic variants in *BRCA1/2* genes which predispose to hereditary breast and ovarian cancer syndrome (HBOC). The prevalence and spectrum of *BRCA1/2* pathogenic variants vary among different populations and are responsible for only approximately 25–50% of the familial risk of BC[2–4]. As DNA sequencing technologies evolved, other cancer susceptibility genes have been discovered, including high-penetrant genes such as *TP53*, *CDH1*, *STK11*, *PTEN* and *PALB2* (> 5 fold cancer relative risk), moderate-penetrant genes such as *CHEK2* and *ATM* (2–5 fold cancer relative risk), and a number of common low-penetrant BC susceptibility loci identified through genome-wide association studies (1–2 fold cancer relative risk)[5–7]. The mutational spectrum of germline mutations in BC predisposition genes have been reported in single populations, with the majority of reports focused on Caucasians from Europe and North America. The population from Southern Hemisphere countries, except for Australia, are underrepresented and understudied in cancer genetic epidemiology research[2].

The Brazilian population has unique ethnic characteristics. People miscegenation is a universal phenomenon, due to globalization and large waves of immigration. Brazil is considered an ethnic “melting pot”, reflecting an admixture of European, Indigenous and Sub-Saharan African people, in addition to immigrants from a large number of European, Asian and Middle Eastern countries. Hence, Brazilian people offer a unique opportunity to advance the understanding of cancer genetic features in a miscegenated population[8].

In Brazil, the majority of the inherited BC studies focused on the analyses of *BRCA1/2* as well as *TP53*, given the relatively high population frequency of the *TP53* R337H (also known as, c.1010G > A, p.Arg337His) founder mutation in people from the South and Southeast regions of Brazil[9]. However, the likelihood of carrying pathogenic mutation in other BC susceptibility genes among *BRCA1/2* and *TP53*-negative patients is largely unexplored.

Recent advances in next generation sequencing (NGS) technology has reduced the cost of massively parallel sequencing, provided to physicians and patients the option of sequencing multiple genes simultaneously and broadened our understanding of the genetic etiology of inherited cancers. Multi-gene panel testing has proved useful as a diagnostic tool for disorders where similar phenotypes can be influenced by multiple genes such as hereditary predisposition to BC, uncovering potentially actionable findings that may be missed by traditional testing paradigms. Several laboratories have released commercial multi-gene panel testing ranging from six to > 100 genes[10]. Panels are cheaper, faster and increase the yield of genetic findings, more than doubling the mutation detection rate in *BRCA1/2*-negative patients with suspected HBOC[11–18].

However, finding a mutation in a gene where the cancer risks and/or management strategies are not known, as well as the identification of higher numbers of variants of uncertain significance (VUS), can make the results cumbersome and challenging for a physician to interpret and guide treatment[10].

These panels have been widely available in Brazil within the past 7 years, but no study has yet assessed the prevalence and mutational spectrum of germline variants in BC susceptibility genes other than *BRCA1/2* and *TP53* in a large cohort of individuals with BC, who were referred for genetic evaluation. Given the rapidly uptake of multi-gene panel testing in clinical practice, these data are urgently needed to inform genetic counseling. In this study, we report the results from 1,662 consecutive individuals with a history of BC who were referred for multi-gene panel testing.

2. Results

2.1. Study population and prevalence of P and LP variants

This study involved a nationwide sample of 1,662 consecutive BC patients who underwent germline genetic testing with a multi-gene cancer panel between 2015 and 2017. Age at BC diagnosis, geographical region where the test was collected and prevalence of P/LP variants are listed in Table 1. Among all patients, 19.4% carried a P/LP variant in at least one gene (**Supplementary Table 1**); panel tests performed in patients ≤ 35 years identified significantly more mutation carriers than in the whole cohort (24.6% vs. 19.4%, p = 0.02).

Table 1

Frequency of pathogenic/likely pathogenic germline variants from 1662 breast cancer patients who underwent multi-gene panel testing, according to age and living country region

Patients with a positive finding: n (%)									
	Total cohort	BRCA1	BRCA2	BRCA1/2	TP53	TP53 R337H	High-penetrant BC genes (n = 8 genes)	Moderate-penetrant BC genes (n = 7 genes)	Multi-gene panel (n = 20–38 genes)
Age at BC diagnosis									
≤ 35 years	390 (23,5)	36 (9,2)	16 (4,1)	52 (13,3)	11 (2,8)	4 (1,0)	68 (17,4)	23 (5,9)	96 (24,6)
≤ 50 years	999 (60,1)	71 (7,1)	40 (4,0)	111 (11,1)	22 (2,2)	14 (1,4)	146 (14,6)	53 (5,3)	216 (21,6)
≤ 65 years	1125 (67,7)	77 (6,8)	46 (4,1)	123 (10,9)	24 (2,1)	16 (1,4)	162 (14,4)	63 (5,6)	236 (21,0)
Total	1662 (100,0)	95 (5,7)	66 (4,0)	161 (9,7)	37 (2,2)	26 (1,6)	219 (13,2)	67 (4,0)	323 (19,4)
Mean, years (SD)	42,9 (11,2)	38,2 (9,3)	42,0 (9,9)	39,7 (9,7)	39,2 (10,5)	42,2 (10,9)	39,8 (9,7)	42,9 (12,3)	40,7 (10,4)
Regions of Brazil									
Southeast	862 (51,9)	52 (6,0)	40 (4,6)	92 (10,7)	22 (2,6)	15 (1,7)	126 (14,6)	36 (4,2)	183 (21,2)
South	293 (17,6)	15 (5,1)	11 (3,8)	26 (8,9)	10 (3,4)	8 (2,7)	41 (14,0)	11 (3,8)	59 (20,1)
North	26 (1,6)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	2 (7,7)	2 (7,7)
Northeast	283 (17,0)	9 (3,2)	7 (2,5)	16 (5,7)	2 (0,7)	1 (0,4)	21 (7,4)	11 (3,9)	38 (13,4)
Central-west	198 (11,9)	19 (9,6)	8 (4,0)	27 (13,6)	3 (1,5)	2 (1,0)	31 (15,7)	7 (3,5)	41 (20,7)

High-penetrant genes: BRCA1, BRCA2, CDH1, NF1, PALB2, PTEN, STK11 and TP53. Moderate-penetrant genes: ATM, BARD1, BRIP1, CHEK2, NBN, RAD51C and RAD51D. Positive findings, carriers of likely pathogenic and pathogenic variants.

Abbreviation: BC, breast cancer; SD, standard deviation.

Overall, 161 patients (9.7%) had a P/LP variant if only mutations in *BRCA1*/2 were considered, 219 patients (13.2%) had a P/LP variant if 8-gene high-penetrant BC panel was considered, and 286 patients (17.2%) had a P/LP variant if the 15-gene high/moderate-penetrant BC panel was considered. Of note, 37 patients (11.5%) had a P/LP variant in known cancer genes traditionally associated with other hereditary cancers, that are not considered as BC predisposing genes.

Mean age at BC diagnosis was 42.9 ± 11.2 years. Age at diagnosis was significantly lower for *BRCA1* mutation carriers (38.2 ± 9.3 years) than in the whole cohort ($p < 0.001$). Age at diagnosis was not associated with the presence of mutations in any other gene when compared to the whole cohort.

The majority of the tests were from patients who inhabited the Southeast region of Brazil (51.9%), which is the most populated in the country. Patients from all other regions were also well represented, except for patients from the North region (1.6%), which is the least densely populated, covered mostly by the Amazon Rainforest.

2.2. Mutation spectrum of P and LP variants

Overall, 341 P/LP variants were identified in 323 patients, comprehending 19.4% of the cohort. As shown in Fig. 1, the three most frequently mutated genes were *BRCA1* (27.9%), *BRCA2* (19.4%) and *TP53* (10.9%), followed by *MUTYH* (9.67%), *ATM* (8.79%), *CHEK2* (6.45%) *and PALB2* (5.27%). In fact, *MUTYH* P/LP variants were detected in 1.98% of the patients, including monoallelic *MUTYH c.1187G > A*, which was detected in 13 out of 1,068 BC patients, as well as in 17 out of 18,919 reference controls (1.22% vs 0.90%; OR = 1.35; 95% CI: 0.77–2.39; p = 0.29) and *MUTYH c.536A > G*, detected in 8 patients and 76 reference controls (0.75% vs 0.40%; OR = 1.86; 95% CI: 0.90–3.87; p = 0.09).

Sixteen patients carried damaging variants in two genes and one patient in three different genes (**Supplementary Table 2**). Of note, one patient presented P/LP variants in both *BRCA1* and *BRCA2*, three patients in *TP53* R337H in association with *BRCA1* c.5266dupC or heterozygous *MUTYH* (n = 2). Additionally, mutated heterozygous *MUTYH*, particularly *MUTYH* c.1187G > A, was the most frequent partner of other mutated genes (such as, *BRCA1*, *BRCA2*, *PALB2* and *TP53*), detected in seven patients (**Supplementary Table 2**).

Both allelic heterogeneity and founder mutations played a role in inherited BC. Allelic heterogeneity among the patients was reflected in the appearance of 179 distinct mutations in 22 genes (**Supplementary Table 3**). Although the mutational profile was heterogeneous, recurrent mutations (detected in three or more individuals) were found in 8 genes: *APC*, *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CHEK2*, *MUTYH*, and *TP53* (**Supplementary Table 1**). The most prevalent *BRCA1* recurrent mutations, which were the European founder mutations c.5266dupC (n = 28) and c.3331_3334delCAAG (n = 13), accounted for 43.2% of all *BRCA1* reported mutations. The European founder *CHEK2* recurrent mutation c.349A > G (n = 7) accounted for 41.2% of all *CHEK2* reported mutations.

The *TP53* R337H is of particular interest because it is a Brazilian founder mutation that presents in 0.3% of the southern and southeastern general populations[20].

2.3. The Brazilian *TP53* R337H founder mutation

Overall, *TP53* was the third most frequently mutated gene and contributed to 2.2% of BC cases in our cohort. *TP53* pathogenic variants were detected in 37 out of 1,662 BC patients and in 21 out of 18,919 reference controls (2.22% vs 0.11%; OR = 20.49; 95% CI: 11.64–39.92; p < 0.001). It is noteworthy that the *TP53* mutations were concentrated in South and Southeast (86.5%; Table 1) compared to the other regions of Brazil (32 vs 5; OR = 2.9; 95% CI: 1.1–7.4; p = 0.03). The Brazilian founder mutation *TP53* R337H accounted for 70.3% of all *TP53* reported mutations and was also concentrated in patients from the South and Southeast regions of Brazil. This variant was detected in 26 out of 1,662 BC patients, as well as in 17 out of 18,919 reference controls (1.56% vs 0.09%; OR = 17.67; 95% CI: 9.21–34.76; p < 0.001) (Fig. 2). Another 10 patients had mutations in the *TP53* DNA binding domain. *TP53* R337H carriers were diagnosed with breast cancer an average of 10 years older than patients who carried *TP53* pathogenic variants within typical DNA-binding domain (42.2 ± 10.9 years vs. 32.3 ± 5.1 years, p < 0.05).

2.4. VUS in Brazilian patients with BC

The overall VUS rate was 46.1% for the entire patient population, with 13.4% having two or more VUS (Fig. 3; **Supplementary Fig. 1**). As expected, the prevalence of VUS increased considerably with the number of genes tested. The chance to detect a VUS was 6.7% if only *BRCA1/2* test was considered. Comparing to a *BRCA1/2* test, this chance was approximately 2 times higher if a 8-gene high-penetrant BC panel was considered (OR = 2.26; 95% CI: 1.78–2.86; p < 0.0001), 7 times higher if a 15-gene high/moderate-penetrant BC panel was considered (OR = 7.19; 95% CI: 5.78–8.93; p < 0.0001), and almost 12 times higher if a multi-gene panel was considered (OR = 11.83; 95% CI: 9.55–14.66; p < 0.0001).

3. Discussion

This is the largest nationwide cohort of Brazilian BC patients who underwent NGS muti-gene panel testing reported to date. The most commonly mutated genes were *BRCA1/2*, which were identified in 10% of the entire cohort and accounted for almost 50% of all P/LP germline variants identified. In accordance with previous research from different countries, the use of a multi-gene panel test doubled the yield of P/LP variants detected, as well as increased in 12 times the chance of finding a

VUS. Most significantly, this study differs from the others because it highlights the important contribution of Li-Fraumeni syndrome (LFS) to inherited BC burden in Brazil, due to the founder mutation *TP53* R337H. It is worth emphasizing that the number of patients carrying this mutation is similar to the number of patients with *BRCA1* c.5266dupC, which is the most prevalent *BRCA1* pathogenic variant in our study.

The estimated frequency in the general population of P/LP *BRCA1/2* mutations is 1:800–1:1000 per gene[21]; however, the prevalence of pathogenic variants in *BRCA1/2* varies considerably between different ethnic groups and geographic areas. In Brazil, there are no large population studies yet, so we do not have reliable estimates of its prevalence in this scenario. The prevalence of *BRCA1/2* pathogenic variants in unselected, under the age of 35 or classified as high-risk BC patients was estimated to be 2.3%[22], 16.5–20.4% and 3.4–22.5%, respectively[23–32] (Table 2). Our unselected cohort probably has the bias of comprehending mainly high-risk patients, as they were probably referred for genetic testing due to suspicion of the attending physician, identified a percentage of patients with a *BRCA1/2* mutation of approximately 10%. The two most prevalent mutations are in accordance with the largest study of the Brazilian population reported to date: *BRCA1* c.5266dupC and *BRCA1* c.3331_3334delCAAG[33,34]. The *BRCA1* c.5266dupC founder pathogenic variant is the most frequently reported in Brazil by several independent studies, but has not been observed elsewhere in South America, with the exception of an Ashkenazi community in Argentina. Notwithstanding, the *BRCA1* c.3331_3334delCAAG was identified in BC patients in Spain and Portugal, as well as in Brazil, Chile, and Colombia. Despite a significant contribution of African ancestry to the genetic pool of some of the populations of Brazil, no recurrent pathogenic variants were traced back to the African continent in our cohort[35].

Table 2
Prevalence of BRCA1/2 mutation in HBOC patients in Brazil

Reference	n	Studied population	BRCA1, n (%)	BRCA2, n (%)	BRCA1/2, n (%)	Screening methodology	BRCA1 covered region	BRCA2 covered region
Gomes *et al.*, 2007[22]	402	unselected BC	6 (1,5%)	3 (0,8%)	9 (2,3%)	OMM + DS	partial	partial
Carraro *et al.*, 2013[24]	54	BC < 35 years	7 (13%)	4 (7,4%)	11 (20,4%)	DS	complete	complete
Encinas *et al.*, 2018[23]	79	BC < 35 years	4 (5,1%)	9 (11,4%)	13 (16,5%)	DS + MLPA	complete	complete
Lourenço *et al.*, 2004[30]	47	high-risk BC	7 (15%)	NA	7 (15%)	DS	complete	NA
Dufloth *et al.*, 2005[26]	31	high-risk BC	1 (3,2%)	3 (9,7%)	4 (12,9%)	OMM + DS	partial	partial
Silva *et al.*, 2014[27]	120	high-risk BC	20 (16,7%)	7 (5,8%)	27 (22,5%)	DS + MLPA	complete	complete
Esteves *et al.*, 2009[33]	612	High-risk (PH ± FH)	19 (2,9%)	3 (0,5%)	21 (3,4%)	OMM	partial	partial
Ewald *et al.*, 2011[29]	137	High-risk (PH ± FH)	7 (5%)	NE	7 (5%)	DS	c.68_69delAG, c.5266dupC	c.5946delT
Felix *et al.*, 2014[30]	106	High-risk (PH ± FH)	9 (8,5%)	0	9 (8,5%)	DS	complete	c.5946delT, c.156_157insAlu
Palmero *et al.*, 2016[31]	18	High-risk (PH ± FH)	0	1 (7,1%)	1 (7,1%)	OMM	partial	partial
Fernandes *et al.*, 2016[34]	349	High-risk (PH ± FH)	49 (14%)	26 (7,5%)	75 (21,5%)	DS + MLPA	complete	complete
Alemar *et al.*, 2017[32]	418	High-risk (PH ± FH)	51 (12,2%)	31 (7,4%)	80 (19,1%)	DS + MLPA	complete	complete
Timoteo *et al.*, 2018[49]	157	High-risk (PH ± FH)	11 (7.0)	5 (3.2)	16 (10.2)	DS	complete	complete
Cipriano Jr *et al.*, 2019[40]	44	High-risk (PH ± FH)	5 (11.4)	7 (15.9)	12 (27.3)	OMM + DS	partial	partial
Bandeira *et al.*, 2021[50]	105	High-risk (PH ± FH)	10 (9.5)	4 (3.8)	14 (13.3)	DS + MLPA	complete	complete
da Costa E Silva Carvalho *et al.*, 2020[41]	95	High-risk (PH ± FH)	13 (13.7%)	4 (4.2%)	17 (17.9%)	DS + MLPA	complete	complete

Reference	n	Studied population	BRCA1, n (%)	BRCA2, n (%)	BRCA1/2, n (%)	Screening methodology	BRCA1 covered region	BRCA2 covered region
Guindalini *et al.* (current study)	1662	BC refered to GT	95 (5.7)	66 (4.0)	161 (9.7)	DS + MLPA	complete	complete

Abbreviations: BC, breast cancer; HP ± HF, personal history and / or family history of breast and / or ovary cancer; GT, genetic testing; MLPA, multiplex ligation- dependent probe amplification; NE, not evaluated; OMM, other molecular methods such as denaturing high performance liquid chromatography (DHPLC), high resolution melting (HRM), protein truncation test (PTT), single-strand conformation polymorphism (SSCP); SD, direct sequencing like Sanger or next generation sequencing (NGS).

In the present series, the two most commonly detected monoallelic *MUTYH* P/LP variants, *MUTYH c.1187G > A* and *MUTYH c.536A > G*, were not associated with breast cancer risk, in accordance with some authors[36] (Out *et al.*, 2012), but not with others (Win *et al.*, 2016)[37]. Damaging variants in the *TP53* gene are very relevant for the Brazilian population. In general, the global prevalence estimates of P/LP *TP53* variants are within the range of one carrier in 3,555–5,476 individuals[38]. In Brazil, the *TP53* R337H variant is estimated to occur in about 2.7 per 1,000 individuals born in southern Brazil[20]. In the 2000s, Brazilian researchers associated the *TP53* R337H variant, which affected the oligomerization domain, with an increased risk of developing adrenocortical carcinomas. Subsequent studies have shown that the same variant could also increase the risk of other cancers, such as BC, but the penetrance was different[39–43]. The *TP53* R337H variant confers a lifetime cancer risk by age 60 years of 80% in females and 47% in males. In comparison, in classic LFS, those with mutation located in typical DNA-binding domain, the cancer risk is 90% in women and 73% in men[44]. The reasons concerning the reduced penetrance of this variant is still controversial and usually associated with its location in the gene and biochemistry stability, which is pH dependent. A recent study showed that an extended haplotype cosegregating the *TP53* R337H and *XAF1* E134* alleles may lead to a more aggressive cancer phenotype than *TP53* R337H alone, acting as a functional modifier by attenuating the transactivation of wild-type and hypomorphic *TP53* variants, such as R337H. Carriers harboring the extended haplotype were more likely to be diagnosed with sarcomas and multiple tumors, nevertheless this association was not observed in BC patients. Further studies are needed to validate these findings and evaluate their implications on genetic counseling and clinical management of *TP53* R337H carriers[45].

BC is the most common malignancy diagnosed in LFS. In Brazil, in high-risk BC patients, the prevalence of *TP53* R337H ranged from 3.4–7.1% in the South/Southeast[46,47] and 0.9% in the Northeast region[30] (Table 3). In a cohort of 815 women affected by BC in southern Brazil who developed the disease before age 45 years, the prevalence of the *TP53* R337H variant was 12.1%[47]. In our cohort, the prevalence of all P/LP *TP53* variants was 2.2%, representing the third most commonly mutated gene among BC patients. The *TP53* R337H variant was responsible for 70.3% all *TP53* mutations identified. Removing the *TP53* R337H variant, it becomes clear that the prevalence of other mutations in *TP53* is low in the Brazilian population, following the same pattern as the worldwide prevalence.

Table 3
Prevalence of TP53 mutation in HBOC patients in Brazil

Reference	n	Inclusion criteria	TP53 covered region	TP53 R337H, n (%)	TP53 mutations, n (%)	Region of Brazil
Palmero *et al.*, 2008[9]	750	Population screening	R337H	2 (0,3%)	2 (0,3%)	South
Assumpção *et al.*, 2008[42]	123	unselected BC	exon 10	3 (2,4%)	3 (2,4%)	Southeast
Gomes *et al.*, 2012[43]	390	unselected BC	R337H	2 (0,5%)	2 (0,5%)	Southeast
Giacomazzi *et al.*, 2014[40]	815	unselected BC	R337H	70 (8,6%)	70 (8,6%)	South/Southeast
Carraro *et al.*, 2013[24]	54	BC < 35 years	complete gene with DS	0 (0,0%)	1 (2%)	Southeast
Giacomazzi *et al.*, 2014[47]	59	high-risk BC	R337H	2 (3,4%)	2 (3,4%)	South
Cury *et al.*, 2014[46]	28	high-risk BC	complete gene with HRM	2 (7,1%)	2 (7,1%)	Southeast
Silva *et al.*, 2014[27]	120	high-risk BC	R337H	3 (2,5%)	3 (2,5%)	Southeast
Felix *et al.*, 2014[30]	106	high-risk BC	R337H	1 (0,9%)	1 (0,9%)	Northeast
da Costa E Silva Carvalho *et al.*, 2020[41]	94	high-risk BC	complete gene with DS	5 (5.3)	6 (6.4)	Southeast
Bandeira *et al.*, 2021[50]	105	high-risk BC	complete gene with DS	1 (0,9)	1 (0,9)	Southeast
Cipriano Jr *et al.*, 2019[40]	44	high-risk BC	R337H	1 (2.3)	1 (2.3)	Southeast
Guindalini *et al* (current study)	1662	BC refered to GT	complete gene with DS	26 (1.6)	37 (2.2)	All

Abbreviations: BC, breast cancer; DS, direct sequencing like Sanger or next generation sequencing (NGS); GT, genetic testing; HRM, high resolution melting.

Thus, these results confirm that inheritance of *TP53* R337H contribute to a significant number of BC cases in Brazil. These findings reaffirm the need for differentiated guidelines for monitoring and risk reduction strategies in patients with hereditary BC in Brazil. The investigation of the *TP53* R337H variant in the Brazilian pre-menopausal patients diagnosed with BC is essential. These patients and their relatives who carry the same variant should receive intensive surveillance which includes at least whole-body magnetic resonance imaging (MRI) and central nervous system MRI according to Toronto protocol[48]. In addition, breast MRI should be offered annually from age 20 years and mammography annually after age 30 years. For these patients, risk-reducing bilateral (adeno)mastectomy should be discussed. For BC patients, mastectomy should be the preferred option in an attempt to avoid radiotherapy. Nonetheless, radiotherapy should be considered when the risk of locoregional recurrence is high.

4. Conclusion

In summary, the largest nationwide cohort of Brazilian BC patients who underwent NGS multi-gene panel testing identified that *BRCA1/2* accounted for almost 50% of all P/LP germline variants. The third most frequently gene mutated was the *TP53* due to the high number of *TP53* R337H carriers in the South and Southeast region of Brazil. The high prevalence of this founder

mutation has a significant impact in our screening and risk-reducing strategies. In addition, the use of multi-gene panel testing increased significantly the chance of finding a VUS.

5. Methods

5.1 Study population

Patients were eligible to participate if they were 18 years of age or older, had a personal diagnosis of BC, and were referred for a commercial multi-gene cancer panel testing at a College of American Pathology (CAP)–accredited laboratory (Mendelics Análise Genômica S.A., São Paulo, SP, Brazil). Informed consent for clinical testing and use of data for scientific purposes was obtained and demographics/personal histories were collected from test requisition forms by the ordering physician. The protocol was approved by the Faculdade de Medicina da Universidade São Paulo (FMUSP) Institutional Review Board. All patient data were anonymized before analysis. The absence of personal information in medical requests were interpreted as "not provided".

5.2 Panel composition

A custom targeted NGS panel was chosen at the discretion of the ordering clinician and ranged from 20 to 38 genes. At least the 20 genes included on the Mendelics curated BC panel (*AKT1*, *ATM*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *FANCC*, *NBN*, *NF1*, *PALB2*, *PTEN*, *TP53*, *RAD51C*, *RAD51D*, *STK11*, *PIK3CA*, *RECQL*) were evaluated in all patients. Among these genes, 15 (high-penetrant: *BRCA1*, *BRCA2*, *CDH1*, *NF1*, *PALB2*, *PTEN*, *STK11* and *TP53*; moderate-penetrant: *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *NBN*, *RAD51C* and *RAD51D*) are referenced in NCCN management guidelines and commonly included in diagnostic BC panels[19]. Analysis of other genes was performed at the discretion of attending physician and included one or more genes from a 38 gene panel, which included *APC*, *ATM*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *EGFR*, *EPCAM*, *FANCC*, *MEN1*, *MET*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *NF1*, *NF2*, *PALB2*, *PMS2*, *POLD1*, *POLE*, *PTEN*, *RAD51C*, *RAD51D*, *RB1*, *RECQL*, *RET*, *STK11*, *TP53*, *WT1*.

5.3. Sequencing and variant interpretation

Genomic DNA was obtained from a buccal swab or peripheral blood sample using standard methods. DNA Sequencing was performed by high-end Illumina platforms (HiSeq 2500 and HiSeq 4000). Base calling was performed using original Illumina tools (bcl2fastq). Bioinformatics pipeline followed Broad Institute best practices (https://gatk.broadinstitute.org/hc/en-us/sections/360007226651-Best-Practices-Workflows). After alignment to the reference genome GRCh37 / UCSC hg19, low quality and duplicate readings were removed, and variants (SNPs/indels) were detected with GATK HaplotypeCaller. Enrichment and analysis concentrated on the coding sequences, flanking intronic regions (± 20bp) and other specific genomic regions previously identified that harbor causing variants. Promoters, untranslated regions and other non-coding regions were not analyzed. Exonic deletions and duplications (CNV) were identified using ExomeDepth, an R package that estimates the number of copies by comparing the reading depth for each target with the mean reading depth for the same target from samples genotyped from the same sequenced library. If a CNV was identified, multiplex ligation-dependent probe amplification (MLPA) assay was employed to confirm the finding. The variants were classified according to algorithms based on machine learning and described with a nomenclature compatible with the norms and guidelines of the American College of Medical Genetics and Genomics (ACMG)/Human Genome Variation Society (HGVS). Variants interpreted as pathogenic (P) and likely pathogenic (LP) were considered positive. All variants were evaluated by a medical geneticist or pathologist or certified oncologist.

5.4. Brazilian genomic database

Reference control data were obtained from the Mendelics Análise Genômica S.A. database, which contains panel and exome sequencing data from 18,919 Brazilian individuals, sequenced as part of various disease-specific genetic tests, excluding samples from cancer cases. Associations of genes with BC were assessed by comparing frequency of mutations in genes of BC patients with those of reference controls.

5.5. Statistical Analysis

Patients characteristics and sequencing results were tabulated, with descriptive statistics including medians, means, and standard deviations for continuous data and proportions with 95% confidence interval (CI) for categorical data are presented. A $\chi 2$ test was used to compare proportions among cohorts and *P* values less than 0.05 were considered significant. Continuous variables were compared by t tests. Odds ratios (OR) and 95% CI were calculated by established methods using SPSS Version 16.

Declarations

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Author contribution

R.S.C.G. and M.A.A.K.F. Conceived and designed the analysis.

R.S.C.G., D.V.V and J.P.F.W.K. Collected the data.

E.F., D.V.V., J.P.F.W.K., F.P.M.M, A.V., D.S. and F.K. Contributed data.

Y.Z., O.I.O, J.P.F.W.K., R.V.M.L, M.A.A.K.F. and R.S.C.G. Performed the analysis.

All authors discussed the results and contributed to the final manuscript.

R.S.C.G. Wrote the manuscript with support from M.A.A.K.F., D.V.V and V.M.R.

Competing interests

The authors declare no competing interests.

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Figures

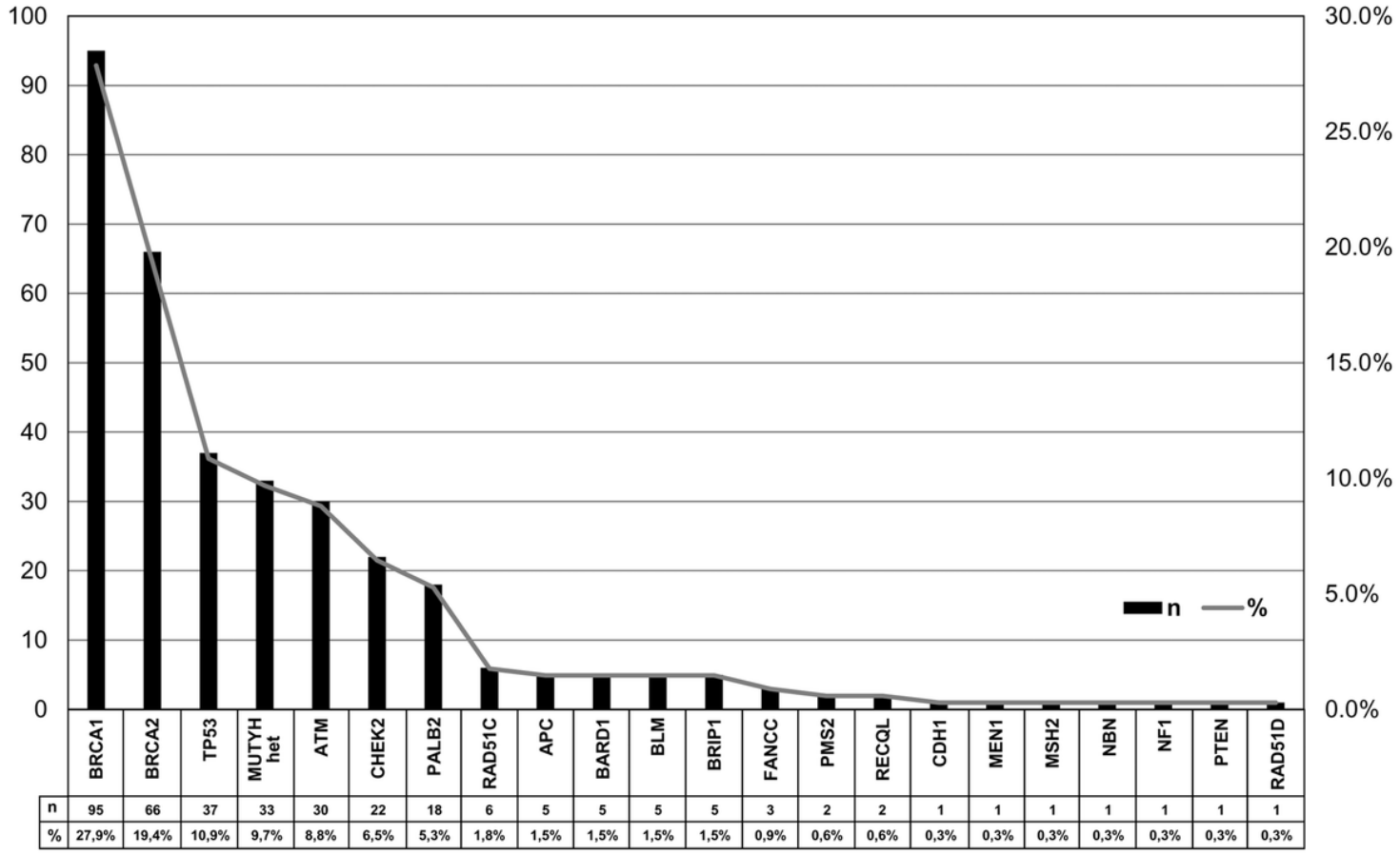

Figure 1

Mutation spectrum of pathogenic and likely pathogenic variants.

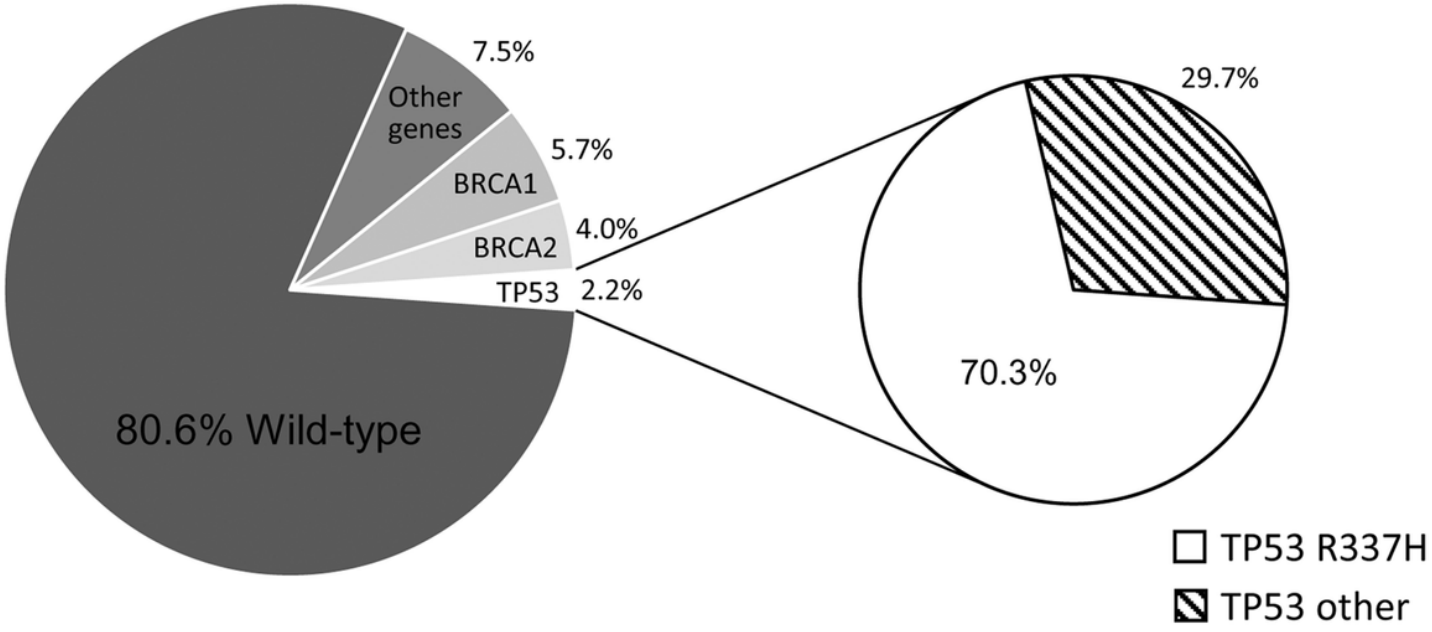

Figure 2

Contribution of TP53 mutation in Brazilian breast cancer patients (n = 1,662). High-penetrant genes: BRCA1, BRCA2, CDH1, NF1, PALB2, PTEN, STK11 and TP53; moderate-penetrant genes: ATM, BARD1, BRIP1, CHEK2, NBN, RAD51C and RAD51D.

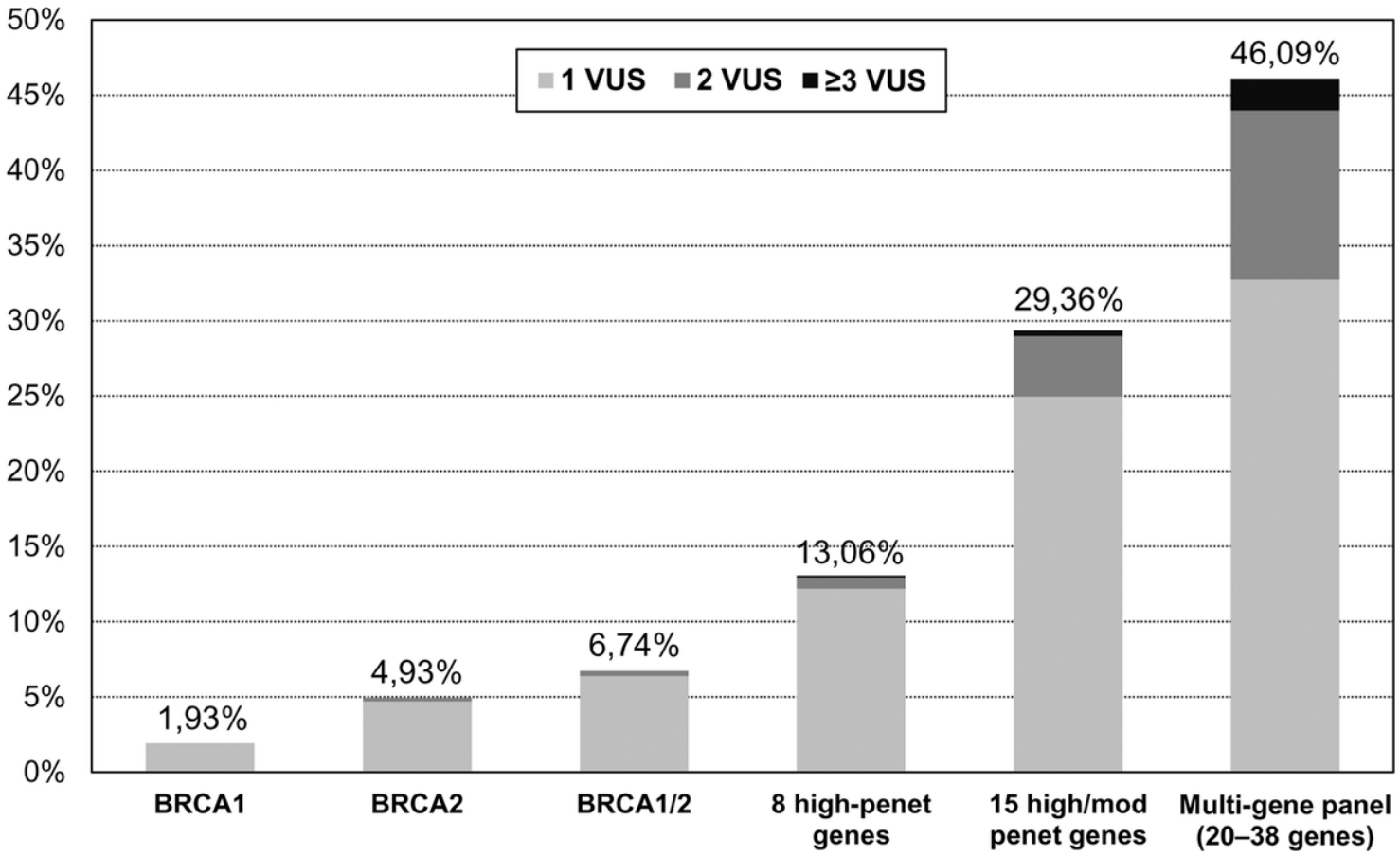

Figure 3

Frequency of variants of unknown significance (VUS). Cumulative fraction of clinical cases with one or more VUS, irrespective of pathogenic variants observed, as the scope of testing increases. High-penetrant genes: BRCA1, BRCA2, CDH1, NF1, PALB2, PTEN, STK11 and TP53; moderate-penetrant genes: ATM, BARD1, BRIP1, CHEK2, NBN, RAD51C and RAD51D.

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