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ORIGINAL ARTICLE

Mutation screening of *TP53, CHEK2* and *BRCA* genes in patients at high risk for hereditary breast and ovarian cancer (HBOC) in Brazil

Nilson Moreira Cipriano Jr[1] · Amanda Marques de Brito[1] · Eneida Santos de Oliveira[1] · Fabiana Castro de Faria[2] · Sara Lemos[2] · Angélica Nogueira Rodrigues[2,3,4] · Débora de Oliveira Lopes[1] · Luciana Lara dos Santos[1]

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Abstract

Background Few studies related to hereditary breast and ovarian cancer syndrome (HBOC) have been conducted in Brazil, and they are restricted to only small areas of the country. Here, we report the mutation profile of *BRCA1/2, CHEK2* and *TP53* genes in a cohort from Minas Gerais state.

Methods These genes from 44 patients at high risk for HBOC were screened through high-resolution melting and/or sequencing. The pathogenicity of the alterations was checked using ClinVar database and bioinformatics programs.

Results In *BRCA* genes we identified 46 variants, 38 without clinical significance and 8 pathogenic mutations including a new pathogenic mutation in *BRCA1* gene (c.4688_4694delACCTGGAinsG). The most prevalent pathogenic mutation was c.4829_4830delTG, in the *BRCA2* gene. This mutation was not described in the Brazilian population up to now and in this study, it was described with a prevalence of 6.8%. The p.R337H mutation in *TP53* gene was found in one patient clinically diagnosed as HBOC and without clinical criteria for Li-Fraumeni syndrome. In *CHEK2* gene, the undescribed variant c.485A > G was found and it presents as probably pathogenic through *in silico* analyses. Pathogenic mutations were found in 29.5% of the patients, 11.3% in *BRCA1*, 15.9% in *BRCA2* and 2.3% in *TP53* gene.

Conclusions Brazilian population is one of the most heterogeneous in the world and the mutational profile knowledge of genes related to HBOC from different regions can contribute to the definition of more cost-effective strategies for the prevention, identification and treatment of cancer.

Keywords HBOC · *BRCA* · *CHEK2* · *TP53* · Brazil

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✉ Luciana Lara dos Santos
llaramg@hotmail.com

Nilson Moreira Cipriano Jr
nciprianojr@gmail.com

Amanda Marques de Brito
amandabrito.bqi@gmail.com

Eneida Santos de Oliveira
eneidaso@hotmail.com

Fabiana Castro de Faria
fabiana.castro@acccom.org.br

Sara Lemos
sara@acccom.org.br

Angélica Nogueira Rodrigues
angelica.onco@uol.com.br

Débora de Oliveira Lopes
debora@ufsj.edu.br

[1] Universidade Federal de São João del Rei (UFSJ), 400 Sebastião Gonçalves Coelho Ave., Chanadour, Divinópolis, MG 35501-296, Brazil

[2] Associação de Combate ao Câncer do Centro Oeste de Minas Gerais (ACCCOM), 500 Topázio st., Niterói, Divinópolis, MG 35500-215, Brazil

[3] Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), 190 Professor Alfredo Balena Ave., Santa Efigênia, Belo Horizonte, MG 30160-011, Brazil

[4] Grupo Brasileiro de Tumores Ginecológicos (EVA), São Paulo, Brazil

Introduction

Breast and ovarian cancers are important causes of cancer morbidity and mortality worldwide. In Brazil, 59,700 new breast cancer cases are expected in 2018, making the disease the most common cancer affecting women. Ovarian cancer is the most lethal gynaecological malignancy, and approximately 6150 cases occur per year in Brazil [1].

There is a large number of genes related to breast and ovarian cancers, but most of them are of moderate or low penetrance; most hereditary ovarian and breast cancers are associated with mutations in high penetrance predisposition genes such as *BRCA1* and *BRCA2* [2]. Approximately, 10–15% of ovarian and 5–10% of breast cancer patients have a *BRCA* germline mutation, characterizing the hereditary breast and ovarian cancer syndrome (HBOC). *BRCA* genes are tumour suppressors involved in genomic integrity; therefore, mutations in these may cause a loss-of-function of the corresponding protein, facilitating the development of a malignant tumour [2]. The diagnosis is frequently made at a young age, and up to 80% of cancers occur before menopause, and bilateral breast cancer is more common in these patients as well [3]. Additionally, mutations in *BRCA* genes may also be associated with pancreatic, stomach, biliary tract and prostate cancers [2]. Recognition of high-risk families is fundamental to early cancer diagnosis and prevention strategies establishment [3].

In Brazil, special attention is given also to *TP53* gene, once p.R337H mutation has a high prevalence in South and Southeast populations [4]. Several studies report that it increases the risk of breast cancer and seems to present the same haplotype in all Brazilian carriers, suggesting a founding effect [4–8]. Pathogenic mutations in *TP53* are the cause of Li-Fraumeni syndrome (LFS/LFL). Due to the high frequency of p.R337H mutation in Brazil and its association with breast cancer, it is relevant to study this in HBOC patients, independently of the criteria for LFS/LFL, especially in the *BRCA*-negative ones [9].

The *CHEK2* gene is also associated with Li-Fraumeni syndrome. This gene has moderate penetrance for breast and/or ovary cancer with a lifetime cumulative risk of over 20% [3]. Studies have shown that 15–33% of *BRCA*-negative patients have mutations in *CHEK2* gene, and therefore, for these individuals, it should be considered [2]. The c.1100delC mutation is the most studied worldwide and its carriers have a cumulative risk of 37% until the age of 70 years to developing breast cancer [10]. The c.470T > C and c.444 + 1G > A mutations in *CHEK2* are also frequently associated with breast cancer and present a relative risk of 1.5 and fourfold for its development [11, 12].

Few molecular *BRCA* studies have been conducted with the Brazilian population, and these have been restricted to a small part of the country. Most of them do not analyse all coding region of *BRCA* genes [13–21]. Recently, we reported an initial study of the *BRCA1* gene in 18 highly selected breast cancer patients at high risk for hereditary breast and ovarian cancer (HBOC) in Minas Gerais state. High-resolution melting (HRM) technology was used [22]. Here, we report the *BRCA1* and *BRCA2* mutation profiles combined with screening for 4 mutations in *TP53* and *CHEK2 genes* in a larger cohort from Minas Gerais state.

Materials and methods

Patients and DNA samples

This work was approved by the Research Ethics Committee of Hospital São João de Deus, Divinópolis, MG, Brazil with favourable opinion number 80/201 on 07/05/2011. Patients with suspected HBOC who presented to this hospital from 2011 to 2014 participated in an interview for family history collection and risk analysis. They were selected according to the HBOC criteria adopted by the American Society of Clinical Oncology (ASCO). Patients received information about HBOC and were invited to be tested for *BRCA* mutations. The ones who agreed to participate signed the consent form. Blood samples were collected from each of them. DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions.

Molecular analyses

Primers were designed or adapted from previous studies for all coding exons of *BRCA1* [22], and *BRCA2* genes (Supplementary Material), and amplicons shorter than 350 bp were analysed by an HRM reaction. Primer 3 V.0.4.0 (http://frodo.wi.mit.edu/. Accessed 3 Apr 2016) and Oligo Analyzer 3.1 (http://www.idtdna.com/analyzer/applications/oligoanalyzer. Accessed 3 Apr 2016) software were used. HRM was performed in triplicate using 5 µl of EvaGreen® Plus qPCR Master Mix (Biotium, USA) or MeltDoctor™ (Thermo Fisher Scientific, USA); 0.2 pmol of each primer and 30 ng of DNA were combined in a final volume of 10 µl. The PCR conditions consisted of an initial step at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s and the specific annealing temperature for 1 min [22 and Supplementary Material]. After qPCR, a denaturation step at 95 °C for 15 s; a renaturation at 55 °C for 15 s, and a final denaturation step at 95 °C for 15 s were used in the HRM reaction. Data were collected in the last step every second by the software, varying by 0.1 °C per second. The analysis was performed by Eco Real-Time PCR System Software version 5.0 and Eco Study software version 5.0. Negative controls were used in each reaction.

Samples with an altered HRM profile were sequenced to characterize the alteration in *BRCA* genes. The reaction consisted of 3.5 µL of BigDye® Terminator v1.1 and v3.1 5X Sequencing Buffer (Applied Biosystems), 1 µL of BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), 10 pmol of each primer (forward and reverse) and 250 ng of PCR product. The volume of the reaction was adjusted with nuclease-free water to 10 µL. The precipitation reaction was performed using an EDTA/ethanol methodology and resuspended in Hi-Di formamide (Applied Biosystems). The DNA was denatured at 95 °C for 5 min, incubated on ice for 2 min and sequenced on a 3500 Genetic Analyzer (Applied Biosystems). Data were analysed in the Variant Reporter® Software V2.0 (Applied Biosystems) by comparing to the reference sequence of the *BRCA1/2* genes (*BRCA1* ID: 672; *BRCA2* ID: 675, National Center for Biotechnology Information).

In the first 18 patients screened by HRM, all coding regions were sequenced to check the sensitivity to the HRM reaction [22]. In the subsequent reactions, positive controls were used, and all samples with an altered profile were sequenced to identify the variants. Ten patients performed molecular testing in private companies.

In *BRCA*-negative patients, point mutations in the *TP53* and *CHEK2* genes were screened. The same protocol was performed for p.R337H in *TP53* gene and c.1100delC, c.470T > C and c.444 + 1G > A in *CHECK2* gene. Primers for p.R337H and c.470T > C / c.444 + 1G > A detection were described previously [23, 24]. Initial screening of the mutation was performed in all *BRCA*-negatives patients by high-resolution melting (HRM) and all altered samples were sequenced to validate HRM and to characterize the alteration. The c.1100delC mutation was screened by direct sequencing and primes designed as described above (Supplementary Material).

Classification of nucleotide alterations

The pathogenicity of the alterations was checked using ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/. Accessed Mar 2017) database. Clinical significance for variants was reviewed by ENIGMA expert panel (Evidence-based Network for the Interpretation of Germline Mutant Alleles). In addition, variants of uncertain significance (VUS) were also predicted by six pathogenicity prediction software programmes. Align-Grantham Variation Grantham Deviation (Align-GVGD) [25]; Polymorphism Phenotyping, Version 2 (PolyPhen-2) [26]; Sorting Intolerant From Tolerant (SIFT) [27]; Mutation Assessor programme, release 2 (MutAss) [28]; Predictor of human Deleterious Single Nucleotide Polymorphisms (PhD-SNP) [29]; and I-Mutant [30] were used for this purpose. The softwares Alternative Splice Site Predictor (ASSP) [31], Human Splicing Finder (HSF) [32], and SplicePort [33] were also used to predict any alteration in splicing sites.

Frequency of variant of uncertain significance in control population

Frequency of variants of uncertain significance found in this study was determined in a healthy population to provide more information about its functional effect. The population analysed composed of 88 individuals (32 men and 56 women) attended in the Municipal Health Support Center of Divinopolis, with no malignant neoplasia and no history of breast and/or ovarian cancer in the family. Blood samples were collected, DNA was extracted and VUS were screened by methods already described before.

Results

Forty-four unrelated female patients with clinical criteria for HBOC were analysed in this study. Thirty-nine of them had breast cancer (four presented with ovarian cancer as a second primary and one also had thyroid cancer), two patients had only ovarian cancers and three individuals had no cancer diagnosis, but strong familial criteria for HBOC.

The average age at cancer diagnosis was 44.5 years old. Twenty-nine percent of cancer patients were younger than 40 years old at the time of their diagnosis. Five breast cancer patients were triple negative, and five had bilateral disease. Ovarian cancer was diagnosed in 13.6% of the probands.

We identified 46 different alterations in *BRCA1/2* genes in these patients. Among them, 38 (82.6%) had no clinical significance, including 15 synonymous variants (presented in the Supplementary Material) and eight mutations (17.4%) were pathogenic. Four mutations in each *BRCA* gene were found in twelve unrelated patients (Tables 1, 2).

The four pathogenic mutations found in the *BRCA1* gene were frameshift. Two mutations were found in exon 11, c.3331_3334delCAAG and c.3756_3759delGTCT. The c.3331_3334delCAAG deletion was found in one patient diagnosed with ovarian cancer at the age of 47 and in her four close relatives with breast cancer. The c.3756_3759delGTCT mutation was found in one patient diagnosed with breast cancer earlier onset of 29 years old. A new pathogenic mutation was detected in exon 16 of *BRCA1* gene, c.4688_4694delACCTGGAinsG, in one patient with breast cancer diagnosis at the age of 63 years. No records about this mutation were found in the consulted literature. The other pathogenic mutation, c.5266dupC was found in exon 20 from two unrelated patients. These patients were diagnosed at 43 and 65 years old, respectively, and both developed breast and ovarian cancers (Table 3).

Table 1 Alterations identified in the *BRCA1* gene and their predicted effect according the ClinVar database (reviewed by ENIGMA expert panel)

Localization	HGVS cDNA	HGVS protein	Het (*n*)	Hom (*n*)	Clinical relevance ClinVar
Exon 11	c.1067A > G	p.Gln356Arg	4	–	Benign
Exon 11	c.2077G > A	p.Asp693Asn	5	–	Benign
Exon 11	c.2612C > T	p.Pro871Leu	15	5	Benign
Exon 11	c.3113A > G	p.Glu1038Gly	11	5	Benign
Exon 11	c.3119G > A	p.Ser1040Asn	2	–	Benign
Exon 11	**c.3331_3334delCAAG**	**p.Gln1111AsnfsTer5**	**1**	–	**Pathogenic**
Exon 11	c.3418A > G	p.Ser1140Gly	1	–	Benign
Exon 11	c.3548A > G	p.Lys1183Arg	11	5	Benign
Exon 11	**c.3756_3759delGTCT**	**p.Ser1253ArgfsTer10**	**1**	–	**Pathogenic**
Exon 11	c.4039A > G	p.Arg1347Gly	1	–	Benign
Exon 15	c.4535G > T	p.Ser1512Ile	1	–	Benign
Exon 16	**c.4688_4694delACCTGGAinsG**	**p.Tyr1563***	**1**	–	**Pathogenic**[a]
Exon 16	c.4837A > G	p.Ser1613Gly	12	5	Benign
Exon 16	c.4956G > A	p.Met1652Ile	1	–	Benign
Exon 20	**c.5266dupC**	**p.Gln1756ProfsTer74**	**2**	–	**Pathogenic**

[a]Variants not reviewed by ENIGMA consortium. Pathogenic mutations are in bold

Synonymous variants are presented in the Supplementary Material

Het heterozygotes, *Hom* homozygotes

Table 2 Alterations identified in the *BRCA2* gene and their predicted effect according the ClinVar database (reviewed by ENIGMA expert panel)

Localization	HGVS cDNA	HGVS protein	Het (*n*)	Hom (*n*)	Clinical relevance ClinVar
5´UTR Éxon 2	c.-26G > A	–	13	–	Benign
Exon 2	**c.2T > G**	**p.Met1Arg**	**2**	–	**Pathogenic**[a]
Exon 3	c.223G > C	p.Ala75Pro	2	–	Benign
Exon 10	c.865A > C	p.Asn289His	4	–	Benign
Exon 10	c.1114A > C	p.Asn372His	15	2	Benign
Exon 10	**c.1310_1313delAAGA**	**p.Lys437IlefsTer22**	**1**	–	**Pathogenic**
Exon 11	c.2971A > G	p.Asn991Asp	4	–	Benign
Exon 11	**c.4829_4830delTG**	**p.Val1610GlyfsTer4**	**3**	–	**Pathogenic**
Exon 11	c.6100C > T	Arg2034Cys	1	–	Benign
Exon 11	c.6220C > A	p.His2074Asn	1	–	Benign
Exon 11	c.6347A > G	His2116Arg	1	–	Benign
Exon 14	c.7397C > T	p.Ala2466Val	1	–	Benign
Exon 15	c.7469T > C	p.Ile2490Thr	2	–	Benign
Exon 22	c.8830A > T	p.Ile2944Phe	1	–	Benign
Exon 24	**c.9154C > T**	**p.Arg3052Trp**	**1**	–	**Pathogenic**
Exon 27	c.10234A > G	p.Ile3412Val	3	–	Benign

[a]Variants not reviewed by ENIGMA consortium. Pathogenic mutations are in bold

Synonymous variants are presented in the Supplementary Material

Het heterozygotes, *Hom* homozygotes

Two missense and two frameshift pathogenic mutations were found in *BRCA2*. The c.2T > G mutation was found in exon 2 in two patients with a strong history of breast and ovarian cancers in their family. One of them had breast cancer at an earlier onset of 23 years old, and the other had an onset at 48 years old. Despite this mutation was not reviewed by ENIGMA expert panel, its pathogenicity was already described by ClinVar (criteria provided of Ambry

Table 3 Clinicopathological characteristics and family history of the patients with pathogenic mutations

Patient	Gene	Alteration	Age at diagnosis	Family history[a]	Tumor	Histological type	Hormonal receptors	Secondary tumor
MP006	*BRCA1*	c.5266dupC	43	3	Breast	IDC	–	Ovarian
MP014	*BRCA1*	c.5266dupC	65	3	Breast	IDC	Triple-negative	Ovarian
MP032	*BRCA1*	c.3756_3759delGTCT	29	2	Breast	IDC	ER+; HER+	–
MP062	*BRCA1*	c.3331_3334delCAAG	47	4	Ovarian	Serous adenocarcinoma	ER+; PR+	–
MP067	*BRCA1*	c.4688_4694delACCTGG AinsG	63	5	Breast	IDC	Triple-negative	–
MP005	*BRCA2*	c.2T > G	48	7	Breast	IDC	ER+; PR+	–
MP018	*BRCA2*	c.2T > G	23	9	Breast	IDC	PR+; PR+; HER+	–
MP016	*BRCA2*	c.1310_1313delAAGA	42	4	Breast	IDC	ER+	–
MP028	*BRCA2*	c.4829_4830delTG	64	4	Ovárian	Serous adenocarcinoma	–	–
MP079	*BRCA2*	c.4829_4830delTG	43	2	Breast	IDC	ER+; PR+	Ovarian
MP083	*BRCA2*	c.4829_4830delTG	29	2	Thyroid	IDC	ER+; PR+	Breast
MP045	*BRCA2*	c.9154C > T	44	5	Breast	IDC	ER+	–
MP003	*TP53*	p.R337H	45	6	Breast	IDC	–	–

IDC invasive ductal carcinoma

[a]Number of cancer cases in the family

– Information not found in medical records

Autosomal Dominant and X-Linked, 2015 and CIMBA Mutation Classification, 2016), BIC (Breast Cancer Information Core, https://research.nhgri.nih.gov/bic/. Accessed Apr 2016) and UMD (Universal Mutation Database, http://www.umd.be/. Accessed Mar 2017). The frameshift mutation c.1310_1313delAAGA in exon 10 was found in a patient diagnosed with breast cancer at 42 years old. The c.4829_4830delTG mutation in exon 11 was found in three patients, two with ovarian cancer and diagnosed at 43 and 64 years old, respectively, and one with diagnosis of thyroid cancer at the age of 29 and breast cancer as a second primary at 39 years old. Finally, the c.9154C > T mutation in exon 24 was found in a patient diagnosed with breast cancer at age 44 with five affected relatives. The clinicopathological characteristics and family history of the patients with pathogenic mutations described here can be found in Table 3 and Sanger sequencing traces in Supplementary Figure.

Despite the strong family history, no pathogenic *BRCA* mutation was found in 72.7% of the patients, but one of them presented the p.R337H mutation in *TP53* gene. The patient with p.R337H mutation was diagnosed with bilateral breast cancer at 45 years of age, and has a family history of breast and bowel cancer. The patient did not present with LFS/LFL criteria according to the national comprehensive cancer network (NCCN) guidelines [3].

In *CHECK2* gene, the c.1100delC, c.470T > C and c.444 + 1G > A mutations were not found in the evaluated patients, but the variant c.485A > G, designated by the ClinVar database as unknown significance was found. Its functional effect was tested in different pathogenicity prediction programs and it was predicted as pathogenic by all of them (Table 4). The effect of this mutation in mRNA was not evaluated once the proband did not want to continue with the research. This variant was not found in the control population as well.

Discussion

Approximately 65,850 new breast and ovarian cancer cases are expected to occur annually in Brazil [1]. Despite 5–10% of all cancer cases being hereditary, until now, few studies tracking *BRCA1* and *BRCA2* genes have been performed in the Brazilian population, which has a large interracial mixing. The characterization of the *BRCA* profile is essential for adequate disease control, once patients from distinct geographical areas may present specific mutation profiles, and due to great impact of these genes on cancer prognosis and response to the therapy [2]. In addition, screening for mutations in other breast or ovarian cancer-related genes, known as high frequency in the population, may also help.

In this study, all coding exons of the *BRCA1* and *BRCA2* genes were analysed in 44 individuals at high risk for HBOC from Minas Gerais state, and 46 alterations were found; 38 were considered with no clinical significance, and 8 were pathogenic. We also traced the p.R337H mutation in the *TP53* gene from all *BRCA*-negative patients (72.7%) and it was found in one patient.

Table 4 In silico predictions regarding the pathogenicity of c.485A > G in *CHEK2* gene

Splice prediction software programmes	Pathogenicity prediction software programmes					
	Align- GVGD	PolyPhen2	SIFT	MutAss	PhDSNP	I-Mutant
	Class	Score	Score	FI score	RI	RI
	Deleterious C65	Deleterious 1.0	Deleterious 0	High 4.13	Disease 8	Decrease 7
ASSP	Alt. Isoform/donor					
HSF	Alt. Isoform/donor	Variant c.485A > G in *CHEK2 gene*				
SplicingPort	Alt. Isoform/donor					

Align-GVGD align-Grantham variation Grantham deviation, using the supplied alignments, class shown (class C65 most likely and C0 less likely); *PolyPhen2* polymorphism phenotyping, version 2, score (the closer to 0, more likely, and closer to 1, less likely); *SIFT* sorting intolerant from tolerant blink, predicted as deleterious if the score is ≤ 0.05 and tolerated if the score is > 0.05; *MutAss* mutation assessor, release 2, predicted as FI (high or medium) or non-FI (low or neutral) and combined score (− 5.76 at 5.37); *PhD-SNP* predictor of human deleterious single nucleotide polymorphisms, prediction and RI shown; *I-Mutant* version 2.0, prediction of disease-associated single point mutation from protein sequence and RI shown, *ASSP* alternative splice site predictor, *HSF* human splicing Finder, *SplicePort* an interactive splice-site analysis tool, *Alt* alternative. All programmes were used in the default online mode

BRCA pathogenic mutations were found in 27.3% of the patients (11.4% in *BRCA1*; 15.9% in *BRCA2*). The pathogenic mutations frequency in *BRCA* genes is in accordance with other similar Brazilian studies, in which all coding regions of *BRCA* genes were analyzed [19–21].

On the other hand, the percentage of pathogenic mutations in *BRCA2* is higher in our study, when compared to other studies conducted in Brazil. Previous studies have found a prevalence of 0.5–10% of mutations in *BRCA2* gene [13–15, 17, 19–21]. We believe that this variation may have occurred because of the number of patients studied, the criteria used to select them or/and the selected region of the country, in addition to the fact that some studies do not track the entire coding region of the *BRCA* genes.

Two patients with *BRCA1* mutation had breast cancer and three patients were diagnosed with both breast and ovarian cancer (two were triple negative). Among *BRCA1* mutations found, the c.3331_3334delCAAG mutation had already been found by previous studies in patients from northeast and southeast region of the Brazil [15, 18, 19, 21]. The c.3756_3759delGTCT mutation is one of the most frequently found in *BRCA1* gene around the world, but no studies were found describing it in Brazil [34]. The Ashkenazi Jewish founder c.5266dupC mutation in *BRCA1* gene is one of the most frequent in Brazilian patients with HBOC, with frequencies ranging from 0.6 to 5.1% in other Brazilian states [14, 15, 19, 20]. In our study, this mutation was found in 16.6% of the *BRCA*-positive patients. The prevalence in this cohort was 4.5%. According to Fernandes et al. [21], the c.3331_3334delCAAG and c.5266dupC *BRCA1* mutations are the most frequent mutations in *BRCA* genes in Brazil and they suggest that these mutations should be screened as the first strategy for genetic testing in Brazil, if the analysis of entire coding region is not possible. In our study, the most prevalent pathogenic mutation was c.4829_4830delTG in the *BRCA2* gene, corresponding to 25% of pathogenic mutations found in *BRCA*-positive patients with a prevalence of 6.8% in the evaluated cohort. We believe that these divergences have occurred probably due to the high miscegenation of the Brazilian population. Here we have also found a new pathogenic *BRCA1* mutation, c.4688_4694delACCTGGAinsG, being described for the first time in the literature. This mutation creates a stop codon at position p.Tyr1563*.

Seven patients had pathogenic mutations in the *BRCA2* gene. Four of them had breast cancer, one had ovarian cancer, one had both and one had thyroid and breast cancer (Table 3). Two missense (c.2T > G and c.9154C > T) and two frameshift (c.1310_1313delAAGA and c.4829_4830delTG) pathogenic mutations were found. Three of these mutations (c.9154C > T, c.1310_1313delAAGA and c.4829_4830delTG) were never described in Brazilian studies. The c.4829_4830delTG mutation was found in 3 unrelated probands with several cancer cases in the family. This mutation has been identified in Korean patients [35] and it is one of the most prevalent mutations in the *BRCA2* gene in patients with a history of breast and ovarian cancer in Pakistan [36]. The c.2T > G mutation was found in two unrelated probands, one with five cases of cancer in the family and the other with three cases. This mutation was already identified in two patients in Brazil [21].

The p.R337H mutation in the *TP53* gene has been reported with high frequency in the south and southeast of Brazil, and is associated with childhood and breast cancer [6–8]. In our study, this mutation was found in 4% of *BRCA* -negative patients at high risk for HBOC from Minas Gerais. The frequency is within the range found in other regions of the south and southeast, from 0.5 to 13% [5, 7]. Assumption et al. [6] studied the frequency of this mutation in 123 cases

of breast cancer and 223 controls in Campinas, southeastern Brazil, and found a frequency of 2.4% in patients and no cases were found in the control population. This study also found a significant association between the presence of p.R337H mutation and breast cancer (p = 0.0442). Gomes et al. [7] performed the screening of p.R337H mutation in Rio de Janeiro, with a population of 390 cases of non-selected breast cancer and 324 controls and a frequency of 0.5% was found in the case population and no cases were found in the control. All patients had breast cancer at a young age and a positive family history.

We found the p.R337H mutation in a woman over 40 years old who was suspected of HBOC and did not meet the clinical criteria for Li-Fraumeni. Because the characteristics of LFS/LFL syndrome are heterogeneous, patients with mutations in *TP53* may not fit within the clinical criteria of LFS/LFL or still have features that overlap the HBOC syndrome. According to Cury et al. [9], the screening of the *TP53* p.R337H mutation concomitantly with the screening of *BRCA1/2* genes in all patients at risk for HBOC should be done. This group performed the screening of the p.R337H mutation in patients with the criteria for HBOC in Ribeirão Preto, SP, and verified its presence in 7.1% of the population. Andrade et al. [37] evaluated data from patients with the p.R337H mutation who did not sufficiently meet the criteria for LFS/LFL and reached the same conclusions, emphasizing the importance of simultaneously screening *TP53* p.R337H and *BRCA*1/2 to avoid sub diagnostics and inadequate genetic counselling.

In addition, the patient in this study carrying the p.R337H mutation has a family history of bowel and male breast cancers. Although breast cancer is in the LFS/LFL spectrum, there is no special attention for male cases as there is for HBOC syndrome, and bowel cancer is not even found in the Li-Fraumeni spectrum [3].

The three most frequent mutations in the *CHEK2* gene (c.1100delC, c.470T > C and c.444 + 1G > A) were not found in this study. This result corroborates with data already published in other regions of Brazil and could be explained by a distinct mutation profile mainly due to the high degree of miscegenation [18–20]. The mutations c.470T > C and c.444 + 1G > A were only screened in Brazil once and they were not found [18]. However, the c.485A > G mutation located near to c.470T > C was found. This mutation is present in exon 4 of the *CHEK2* gene, and leads to the exchange of the negatively charged aspartate amino acid by minor and hydrophobic glycine in the oligomerization domain of the *CHEK2* protein, crucial for functional dimerization [38]. This variant is not described in the literature and, accordingly to the performed analyses described in our study, it has a high probability of being pathogenic. In addition to being able to disrupt protein dimerization, our bioinformatics studies show that this mutation seems to create an aberrant splicing site. The c.485A > G mutation was also not found in the healthy control population studied, which reinforces the idea that it may have a detrimental effect.

Lastly, we observed that, among six patients with ovarian cancer, five presented pathogenic *BRCA* mutation, and in the other one we found a probably pathogenic mutation, c.485A > G in *CHEK2* gene, not described in the literature up to now. Besides, 35.3% of the patients without a pathogenic mutation presented 10–17 alterations assigned as benign/little clinical significance. Studies have suggested that multiple low-risk variants presented in one individual can actually increase significantly the risk of cancer for the carrier [2, 39]. SNPs may influence the activity of the protein or modify its affinity for substrates when located in highly conserved regions of the gene designed to interact with DNA and with other proteins. Because HBOC is a complex disease, variants present in other biologically related genes may act in concomitance, and may contribute to the increased risk of cancer [39].

Concluding, in this study, we present the *BRCA* profile together with the screening for mutations in *TP53* and *CHEK2* genes from patients at high risk for HBOC from Minas Gerais state, Brazil. A new pathogenic mutation in *BRCA1* gene and a probably pathogenic mutation in *CHEK2* gene were found and thirteen patients had their pathogenic mutations identified. The screening of the first degree proband's relatives with known pathogenic mutation was performed to trace strategies of early cancer tracking and to reduce cancer risk to these individuals.

For the implementation of successful molecular tests in clinical practice, it is important to understand the interethnic and intraethnic genetic variability of each population. Since the Brazilian population is one of the most heterogeneous in the world, the mutational profile knowledge from different country regions can contribute to define more cost-effective strategies for HBOC identification, prevention and cancer treatment.

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Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Human and animal rights This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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