The germline mutational landscape of BRCA1 and BRCA2 in Brazil

Edenir Inêz Palermo, Dirce Maria Carraro, Barbara Alemar, Miguel Angelo Martins Moreira, Andréa Ribeiro-dos-Santos, Kiyoko Abe-Sandes, Henrique Campos Reis Galvão, Rui Manuel Reis, Cristiano de Pádua Souza, Natália Campacci, Maria Isabel Achatz, Rafael Canfield Briantese, Maria Nirvana da Cruz Formiga, Fabiana Baroni Makedissi, Fernando Regla Vargas, Anna Cláudia Evangelista dos Santos, Hector N. Seuanez, Kelly Rose Lobo de Souza, Cristina B. O. Netto, Patrícia Santos-Silva, Gustavo Stumpf da Silva, Rommel M. R. Burbano, Sidney Santos, Paulo Pimentel Assumpção, Izabel Maria Monteiro Bernardes, Taisa Manuela Bonfim Machado-Lopes, Thais Ferreira Bomfim, Maria Betânia Pereira Toralles, Ivana Nascimento, Bernardo Garicochea, Sergio D. Simon, Simone Noronha, Fernanda Teresa de Lima, Anisso Marques Chami, Camila Matzenbacher Bittar, Jose Bines, Osvaldo Artigalas, Maria Del Pilar Esteves-Diz, Tirzah Braz Petta Lajus, Ana Carolina Leite Vieira Costa Gifoni, Rodrigo S. C. Guindalini, Terezinha Sarquis Cintra, Ida V. D. Schwartz, Príclela Bernardi, Diego Miguel, Sonia Tereza dos Santos Nogueira, Josef Herzog, Jeffrey N. Weitzel & Patricia Ashton-Prolla.

The detection of germline mutations in BRCA1 and BRCA2 is essential to the formulation of clinical management strategies, and in Brazil, there is limited access to these services, mainly due to the costs/availability of genetic testing. Aiming at the identification of recurrent mutations that could be included in a low-cost mutation panel, used as a first screening approach, we compiled the testing reports of 649 probands with pathogenic/likely pathogenic variants referred to 28 public and private health care centers distributed across 11 Brazilian States. Overall, 126 and 103 distinct mutations were identified in BRCA1 and BRCA2, respectively. Twenty-six novel variants were reported from both genes, and BRCA2 showed higher mutational heterogeneity. Some recurrent mutations were reported exclusively in certain geographic regions, suggesting a founder effect. Our findings confirm that there is significant molecular heterogeneity in these genes among Brazilian carriers, while also suggesting that this heterogeneity precludes the use of screening protocols that include recurrent mutation testing only. This is the first study to show that profiles of recurrent mutations may be unique to different Brazilian regions. These data should be explored in larger regional cohorts to determine if screening with a panel of recurrent mutations would be effective.
requests for materials should be addressed to P. A.-P. (email: pprolla@gmail.com).

Edenir Inêz Palmero, Dirce Maria Carraro and Barbara Alemar contributed equally to this work. Correspondence and requests for materials should be addressed to P.-A.-P. (email: pprolla@gmail.com).

Brazil.

Catarina, Florianópolis, Brazil.

Hospital São Carlos, Fortaleza, Brazil.

Aconselhamento Genético, Centro de Oncologia Avançado/CECAN, Universidade Federal do Rio Grande do Norte - Hospital Liga Contra o Câncer, Natal, Brazil. 21Rede D’Or (Fujiday and OncoStar), Fortaleza, Brazil. 22Oncocentro, Hospital São Carlos, Fortaleza, Brazil. 23CLION, CAM Group, Salvador, Brazil. 24Laboratório Genoma, Vitória, Brazil. 25Serviço de Genética Médica do Hospital Universitário, Divisão de Clínica Médica, Universidade Federal de Santa Catarina, Florianópolis, Brazil. 26Hospital Universitário Professor Edgard Santos, Serviço de Genética Médica, Universidade Federal da Bahia, Salvador, Brazil. 27Departamento de Oncogenética, Oncoclin de Manaus, Manaus, Brazil. 28Department of Population Sciences, Division of Clinical Cancer Genomics - City of Hope, Duarte, USA.

Edenir Inêz Palmero, Dirce Maria Carraro and Barbara Alemar contributed equally to this work. Correspondence and requests for materials should be addressed to P.-A.-P. (email: pprolla@gmail.com).

Results

A total of 649 reports of pathogenic/likely pathogenic variants were retrieved from 28 centers in 11 different Brazilian States. As shown in Fig. 1, the majority of reports was obtained from the State of São Paulo (60.1%), which is also the State with the largest number of participating centers (N = 7). The second largest number of reports was obtained from the State of Rio Grande do Sul (16.5%).

As shown in Table 1, 126 distinct pathogenic BRCA1 variants were identified among 441 probands, corresponding to 68% (441/649) of all reported mutations. Among these, a subset of 33 distinct BRCA1 mutations corresponded to 73.4% of all variants identified in this gene. The nine most prevalent BRCA1 mutations accounted for 50.3% of all BRCA1 reported mutations, and among these, the European founder mutation c.5266dupC (formerly known as 5382insC) was the most common, corresponding to 20.2% of all variants found in BRCA1. In BRCA2, 103 distinct variants were identified in 208 probands, corresponding to 32% of all individuals tested (Table 2). The mutational profile of BRCA2 was more heterogeneous, since non-recurring mutations (those seen only once) were more common (35.1%) than in BRCA1 (15.4%). Moreover, a higher frequency of novel variants was identified in BRCA2 (17/103) when compared to BRCA1 (9/126). Figures 3 and 4 show all reported BRCA1 and BRCA2 mutations, respectively, including LGR in both genes. Detailed information about BRCA1 and BRCA2 mutations (predicted protein change, rs number and overall frequency) are summarized in the Supplementary Dataset.
Although the most common mutation, \textit{BRCA1} c.5266dupC, was reported in all geographical regions, some recurrent \textit{BRCA1} mutations (detected in three or more individuals) seem to be unique to a particular Brazilian State. The variants c.188 T\textgreater A, c.2405_2406delTG, c.3916_3917delTT, c.689_692delAGAC, c.4287C\textgreater A, and c.5123C\textgreater A were reported exclusively among individuals recruited from the State of São Paulo (Southeastern region). In addition, the c.1039_1040delCT and c.1039delC variants were reported exclusively in the State of Pará (Northern region), while c.3598C\textgreater T and c.5177_5180delGAAA were only reported in pathogenic mutation carriers from the State of Rio Grande do Sul (Southern Region). No similar trends were observed among \textit{BRCA2} recurrent mutations.

When considering all \textit{BRCA1} and \textit{BRCA2} mutations seen in three or more individuals, a subset of 51 variants (33 in \textit{BRCA1} and 18 in \textit{BRCA2}) accounted for 67% of all reports. In a more stringent scenario, mutations seen in four or more individuals, totaling 30 variants (23 in \textit{BRCA1} and 7 in \textit{BRCA2}) corresponded to 57.3% of all mutations.

**Discussion**

Many factors affect the probability of developing breast or ovarian cancer, but no predictor is as determinant and prevalent as the inheritance of a \textit{BRCA} mutation. There are several clinical management options for individuals harboring \textit{BRCA} mutations, including risk reducing surgeries (bilateral risk-reducing mastectomy, salpingo-oophorectomy)\textsuperscript{15}, chemoprevention\textsuperscript{16} and intensive surveillance with annual breast magnetic resonance imaging\textsuperscript{17}. Several studies have demonstrated that, after identifying a \textit{BRCA}-mutation carrier, genetic counseling and testing of at-risk individuals results in increased surveillance and use of risk-reduction strategies ultimately leading to primary or secondary prevention of cancer and improved outcomes in carriers\textsuperscript{18}. Despite these benefits there is limited availability of genetic testing in Latin American countries, including Brazil\textsuperscript{14,19}.

Low cost screening panels including recurrent \textit{BRCA} pathogenic variants (e.g. Ashkenazi Jewish Panel) have been used in certain countries/populations as an initial approach to overcome technical and economical
**Mutations identified in one proband (N = 68; 15.4%)**

| Mutation           | Identified in 1 proband | Identified in 2 probands (N = 25; 11.3%) | Identified in 3 or more probands (N = 33; 73.4%) | N and (%) |
|--------------------|-------------------------|------------------------------------------|-------------------------------------------------|-----------|
| c.65T>G (5)        | c.3534delC (5)          | c.1A>G (89)                              | c.4996delC (89)                                 | 8 (20.2)  |
| c.190T>C (5)       | c.3544C>T (5)          | c.66dupA (45)                           | c.3331_3334delCAAG (45)                        | 10 (2.2)  |
| c.273_274delTG     | c.3627dupA (19)        | c.244_245insA (19)                      | c.68_69InsA (19)                               | (4.3)     |
| c.302-1G>A (11)    | c.3770_3771delADG (11) | c.791_794delGTTC (11)                   | c.211A>G (11)                                  | (3.0)     |
| c.442-2A>G (5)     | c.3967C>T (5)          | c.1088delA (14)                          | c.5074 + 2T>C (14)                             | (3.0)     |
| c.450delC (5)      | c.4065_4068delTCAAG (5)| c.1912delG (11)                         | c.470_471delCTC (11)                           | (2.5)     |
| c.514delC (5)      | c.4096 + 1G>A (5)      | c.2037delinsCC (10)                     | c.1687C>T (10)                                 | (2.3)     |
| c.679G>T (5)       | c.4117G>T (5)          | c.2038_2039insCC (9)                    | c.4675 + 1G>A (9)                              | (2.0)     |
| c.718C>T (5)       | c.4185G>A (5)          | c.2389_2390delGA (8)                    | c.4484G>T (8)                                  | (1.8)     |
| c.763G>T (5)       | c.4327C>T (5)          | c.2477_2478delCA (6)                    | c.181T>G (6)                                   | (1.4)     |
| c.824_825ins10     | c.4357 + 1G>A (5)      | c.2727_2730delTCAAG (6)                | c.798_799delTT (6)                             | (1.4)     |
| c.833_834insA (5)  | c.4357 + 1G>C (5)      | c.3018_3021delTTCA (6)                  | c.5062_5064delGTT (6)                          | (1.4)     |
| c.850C>T (5)       | c.4625_4626delICT (5)  | c.3228_3229delICT (5)                   | c.188T>A (5)                                   | (1.1)     |
| c.869T>G (5)       | c.4663delA (5)         | c.3257T>G (5)                           | c.1039_1040delICT (5)                          | (1.1)     |
| c.1115G>A (5)      | c.4675 + 1G>T (5)      | c.3403C>T (5)                           | c.2405_2406delTG (5)                           | (1.1)     |
| c.1123_1124delinsA  | c.4688_4694delinsG (5)| c.3640G>T (5)                           | c.3598C>T (5)                                  | (1.1)     |
| c.1327A>T (5)      | c.4689C>G (5)          | c.3627dupA (5)                          | c.3817C>T (5)                                  | (1.1)     |
| c.1340_1341insG     | c.4712_4716delICTCT (5)| c.3764dupA (5)                          | c.3916_3917delTT (5)                           | (1.1)     |
| c.1471C>T (5)      | c.4736_4739delCTTC (5)| c.4754_4755delCA (5)                    | c.4165_4166delAG (5)                           | (1.1)     |
| c.1504_1508delTTAAA| c.4941delC (5)         | c.5084_5085delIT (5)                    | c.4964_4982del (5)                             | (1.1)     |
| c.1556delA (5)     | c.4987_3C>G (5)        | c.5444G>A (5)                           | c.5177_5180delGAAG (5)                         | (1.1)     |
| c.1612C>T (5)      | c.5095C>T (5)          | c.5463_5464insT (5)                     | c.5251C>T (5)                                  | (1.1)     |
| c.1789G>T (5)      | c.5161delIC (5)        | Deletion exon 1–2 (4)                   | c.4383C>T (4)                                  | (0.9)     |
| c.1823delA (5)     | c.5267_5268insC (5)    | Deletion exon 5–7 (3)                   | c.689_692delGAC (3)                            | (0.7)     |
| c.1962dupG (5)     | c.5445G>A (5)          | Deletion exon 21–23 (3)                 | c.441 + 2T>A (3)                               | (0.7)     |
| c.2176_2177delICT  | c.5509T>C (3)          | c.1039delC (3)                          | c.3018_3021delTTCA (3)                         | (0.7)     |
| c.2217dupA (3)     | Deletion exon 3 (3)    | c.330dupA (3)                           | c.1961delA (3)                                 | (0.7)     |
| c.2250dupC (3)     | Deletion exon 3–4 (3)  | c.1961delA (3)                          | c.1961delA (3)                                 | (0.7)     |
| c.2331T>G (3)      | Deletion exon 8 (3)    | c.4287C>A (3)                           | c.1961delA (3)                                 | (0.7)     |
| c.2722G>T (3)      | Deletion exon 9–19 (3) | c.5030_5033delICTAA (3)                | c.1961delA (3)                                 | (0.7)     |
| c.2834_2836delinsC  | Deletion exon 14–16 (3)| c.5096G>A (3)                           | c.5096G>A (3)                                  | (0.7)     |
| c.2910dupA (3)     | Deletion exon 16–17 (3)| c.5123C>A (3)                           | c.5123C>A (3)                                  | (0.7)     |
| c.3041T>C (3)      | Deletion exon 18–19 (3)| Deletion exon 19 (3)                    | Deletion exon 19 (3)                           | (0.7)     |
| c.3329T>A (3)      |                          |                                          | Deletion exon 19 (3)                           | (0.7)     |
| c.3320_3273delACCT |                          |                                          | Deletion exon 19 (3)                           | (0.7)     |

Table 1. Reported mutations in BRCA1, showing 126 distinct mutations identified in 441 unrelated individuals. Mutations in bold are novel (not described in ClinVar, BRCA Share, LOVD, ARUP or BRCA Exchange database) and underlined mutations were described in other database but not in ClinVar. Frequencies and proportions (%) in each column correspond to the fraction of each group among all BRCA1 variants identified (N = 441). See Supplementary Dataset for detailed information.

restrictions that still exist for comprehensive BRCA1 and BRCA2 testing. Most of the populations where this strategy is used show few mutations occurring at a high frequency, often due to founder effects. Thus, the development of such panels depends on a deep knowledge of the mutational spectrum of the target population and the presence of a relatively small number of recurrent mutations explaining a significant proportion of cases. This strategy has been proposed, for instance, for Hispanic breast and/or ovarian cancer families (with predominantly Mexican origin) where nine recurrent variants account for 53% of all detected BRCA mutations. For this strategy to show few mutations occurring at a high frequency, often due to founder effects. Thus, the development of such panels depends on a deep knowledge of the mutational spectrum of the target population and the presence of a relatively small number of recurrent mutations explaining a significant proportion of cases. This strategy has been proposed, for instance, for Hispanic breast and/or ovarian cancer families (with predominantly Mexican origin) where nine recurrent variants account for 53% of all detected BRCA mutations. For this population, a low-cost multiplex PCR-based panel (HISPANEL) was developed and subsequently estimated to identify up to 75% of all true Mexican BRCA mutations. The pattern of highly recurrent mutations is also seen in other Latin American countries: Bahamas (six recurrent mutations correspond to 89.4% of all carriers), Colombia (three recurrent mutations correspond to 89.4% of all carriers) and Peru (three recurrent mutations correspond to 84.6% of all carriers). However, this striking pattern of recurrent mutations seen in several Latin American populations will likely not be effective, because there does not seem to be a significant overlap of recurrent mutations among different Latin American populations. These results are not surprising due to vary distinct population migration waves and therefore genetic admixture background of Brazil in comparison with the other Latin American countries.
Knowledge about the germline mutational spectrum among Brazilian HBOC patients is limited. Only five studies have performed comprehensive BRCA mutation testing (using gene sequencing and LGR analysis) to date\textsuperscript{25–29}, corresponding to only 1,041 individuals tested, among a Brazilian population of over 207 million people\textsuperscript{30}. Most studies have focused on specific mutations, or screened only a few regions of \textit{BRCA1} and/or \textit{BRCA2} (summarized in the Supplementary Dataset). To our knowledge, this is the largest comprehensive description of the spectrum of germline \textit{BRCA} mutations in different geographical Brazilian regions.

Most of the mutations reported previously in smaller Brazilian studies, involving the analysis of only certain gene regions, have also been identified in our cohort, but it is noteworthy that some of the previously reported recurrent mutations are completely absent in this dataset. The most striking example is the \textit{BRCA1} ins6Kb rearrangement, which was reported by Esteves \textit{et al.}\textsuperscript{31} in seven carriers, five of whom were from Rio Grande do Sul State. However, in our cohort we did not identify this rearrangement in any patient, even considering that Rio Grande do Sul was the second State in terms of the number of reported carriers (N = 107). The \textit{BRCA1} 6 kb insertion can be detected by routine LGR testing through MLPA, and although we cannot assure that all probands were subjected to MLPA analysis, we can expect that most of them with negative sequencing results were also investigated for LGRs, since patients from the private healthcare setting and also those enrolled in research studies are routinely tested for LGR by MLPA. Indeed, a recent study from Alemar \textit{et al.} reported LGR data from 351 HBOC probands from the same

| Mutations identified in one proband (N = 73; 35.1%) | Mutations identified in two probands (N = 12; 11.5%) | Mutations identified in three or more probands (N = 18; 53.4%), N and (%) |
|--------------------------------------------------|-------------------------------------------------|-----------------------------------------------------|
| c.298A>T | c.5753delA | c.658_659delGT | 20 (9.6) |
| c.738delT | c.5782G>T | c.1337T>A | 15 (7.2) |
| c.956dupA | c.5800C>T | c.4829_4830delTG | 11 (5.3) |
| c.1128delT | c.5857G>T | c.5164_5165delAG | 10 (4.8) |
| c.1238delT | c.6243_6246del | c.5681dupA | 8 (3.8) |
| c.1588T>T | c.6381_6382insTT | c.7580_7583dupTAGG | 7 (3.4) |
| c.1792delA | c.6418_6419insTGAA | c.7806-2A>G | 7 (3.4) |
| c.1796_1800delCTTAT | c.6443_6444delCT | c.9097dupA | 3 (1.4) |
| c.2167delA | c.6468_6469delTTC | c.9098_9099insA | 3 (1.4) |
| c.2505dupA | c.6611delC | c.9401delG | 3 (1.4) |
| c.2701delIC | c.6752dupA | c.9481T>T | 3 (1.4) |
| c.2845delT | c.7007G>A | Deletion exon 2 | 3 (1.4) |
| c.3046G>T | c.7060C>T | c.5682C>G | 3 (1.4) |
| c.3195_3198delTAAT | c.7180A>T | Deletion exon 1 | 3 (1.4) |
| c.3264dupT | c.7618-2A>G | c.6952C>T | 3 (1.4) |
| c.3847_3848delAT | c.7679_7680delTT | c.7987delG | 3 (1.4) |
| c.3879_3880delAT | c.7738C>T | c.8488-1G>A | 3 (1.4) |
| c.3975_3978dupTGCT | c.8023A>G | c.9004G>A | 3 (1.4) |
| c.4005dupA | c.8195T>G | 3 (1.4) |
| c.4006_4007insA | c.8247_8248delGA | 3 (1.4) |
| c.4131_4132insTGAGGA | c.8489G>A | 3 (1.4) |
| c.4222C>T | c.8548_8551delGAAG | 3 (1.4) |
| c.4284dupT | c.8695C>T | 3 (1.4) |
| c.4535delG | c.8713delT | 3 (1.4) |
| c.4962T>A | c.8754+4A>G | 3 (1.4) |
| c.4963delT | c.8878C>T | 3 (1.4) |
| c.4968_4969insGT | c.9006delA | 3 (1.4) |
| c.4979_4980delCT | c.9076C>T | 3 (1.4) |
| c.5158_5159insA | c.9117G>A | 3 (1.4) |
| c.5197_5198delTTC | c.9154C>T | 3 (1.4) |
| c.5217_5218insA | c.9282_9397del | 3 (1.4) |
| c.5351delA | c.9371A>T | 3 (1.4) |
| c.5351dupA | c.9699_9702delTATG | 3 (1.4) |
| c.5616_5620delGTAA | Deletion exon 13 | 3 (1.4) |
| c.5621_5624delTCAA | Deletion exon 14 | 3 (1.4) |
| c.5641_5644delAAAT | Deletion exon 25 | 3 (1.4) |
| c.5644_5647delTCAA | 3 (1.4) |

Table 2. Reported mutations in BRCA2, showing 103 distinct mutations identified in 208 unrelated individuals. Mutations in bold are novel (not described in ClinVar, BRCA Share, LOVD, ARUP or BRCA Exchange database). Frequencies and proportions (%) in each column correspond to the fraction of each group among all BRCA2 variants identified (N = 208). See Supplementary Dataset for detailed information.
Brazilian State where the previous cases harboring the 6 kb insertion were reported originally, and the **BRCA1** 6 kb insertion was not detected, suggesting a very low frequency of this LGR in probands with the HBOC phenotype.

Among all distinct mutations identified, 11.8% were novel, corresponding to 4.6% of all carriers and highlighting the heterogeneity of our population. The identification of novel mutations linked to HBOC is a vital information that should be shared with established mutation databases, in order to become useful for interpreting further tests and to answer questions about the association between a variant and phenotype.

Overall, our data show a significant molecular heterogeneity among the **BRCA1** and **BRCA2** mutations identified, and a similar profile of type and molecular consequence of pathogenic variants in both genes. In addition, **BRCA1** mutations were more frequent than **BRCA2** mutations, which is in agreement with previous data showing this same proportion of mutations between both genes among women from different ethnicities, except Asians. Also, similar to previous report, the rate of large genomic rearrangements did not exceed 5% of all mutations. However, it is remarkable that 34.3% of all LGR reported here correspond to the Portuguese founder mutation **BRCA2** c.156_157insAlu.

Although significant, this frequency may still be an underestimation, since until very recently the detection of this particular mutation, which requires a specific PCR reaction, was not carried out by most commercial laboratories. Recently (July 2016), MRC-Holland included an extra probe that detects the wild-type sequence of this region in its **BRCA2** MLPA kits (P090 version B1 and P45 version C1), allowing the detection of this variant during MLPA testing. This simple modification is expected to increase significantly the detection rate of c.156_157insAlu in Brazilian HBOC patients. The probands with c.156_157insAlu identified here were from the States of Minas Gerais (1), Rio de Janeiro (3) Rio Grande do Sul (2) and São Paulo (5) but screening for this LGR should be done for patients regardless of State of origin.

In the current HBOC genetic testing landscape, where most laboratories have migrated to next generation sequencing (NGS), analysis workflows allow the filtering of many types of mutations, including the exclusion of synonymous variants. In our study, we have identified two pathogenic synonymous mutations, and this finding highlights the importance of careful evaluation of each **BRCA** variant detected. It is widely known that synonymous substitutions can alter splicing accuracy, creating or destroying a native donor or acceptor splice site, but they can also modify translation fidelity, **mRNA** structure and protein folding. Indeed, both pathogenic synonymous variants identified in this study

**Figure 3.** Circos plot showing the distribution of all reported **BRCA1** mutations. Point mutations and small deletions and insertions are shown around in the outermost ring, which represents the **BRCA1** exons. The number between brackets correspond to the number of mutation carriers. Each reported LGR is represented by dashed blocks in the three intermediate rings, while the innermost ring represent the **BRCA1** domains.
disrupt splice donor sites, leading to exon skipping. The G nucleotide of \( \text{BRCA1} \) c.4185G>A represents the last nucleotide of exon 11 (according to LRG nomenclature, formerly known as exon 12), and is conserved in 86% of the splice sites in mammals\(^{36}\). This variant lead to an aberrant transcript lacking exon 12\(^{37}\). Similarly, the \( \text{BRCA2} \) c.9117G>A leads to a complete deletion of exon 23\(^{38}\) and produces a frameshift effect similar to other deleterious mutations\(^{39}\). The process of evaluating variant significance should include multiple databases, as four mutations reported here were not described in ClinVar, although classified as clearly pathogenic in other databases. Finally, even variants described in one database should have their significance confirmed in other databases, especially if classified as variants of uncertain significance (VUS). As an example, using an \textit{ex vivo} assay based on a splicing reporter minigene, Brandão \textit{et al.}\(^{40}\) demonstrated that the \( \text{BRCA1} \) c.4987-3C>G variant leads to the skipping of exon 17. However, it remains classified as VUS in ClinVar and it is not reported in other databases.

In this study, we have attempted to compile pathogenic and likely pathogenic \( \text{BRCA1} \) and \( \text{BRCA2} \) variants identified in the main Genetic Cancer Risk Assessment centers in Brazil. Although this report in fact is the most comprehensive to date, both in number of mutations reported, as well as in number of centers/regions of the country included, many limitations must be considered when analyzing the results. We were unable to obtain information on the birthplace for most of the carriers, which would have been more informative than the center where the genetic test was performed. Therefore, data on geographical location should be interpreted with caution. In fact, among some of the individuals tested in the State of São Paulo we were able to identify residents from the Midwest States. This is not unexpected since the paucity of clinical and laboratory personnel trained in clinical cancer genetics in Brazil, results in a pattern of patients with suspected hereditary cancer being referred to testing from different parts of the country to only a few reference centers very distant from their residence place. Moreover, it might be possible that the inclusion of data from point mutation analysis could increase the detection and, consequently, the reporting of a few specific mutations. However, our data regarding the most frequently reported mutations is in agreement with previous Brazilian studies that performed full \( \text{BRCA1} \) and \( \text{BRCA2} \) sequencing and MLPA. These studies show, for example, that the \( \text{BRCA1} \) c.5266dupC is the most prevalent mutation across distinct regions of Brazil, which was also the case in our study\(^{26,27,29}\).
We confirm that there is significant molecular heterogeneity in the BRCA1 and BRCA2 genes among Brazilian carriers. Although our findings suggest that this heterogeneity precludes the use of screening protocols that include recurrent mutation testing only, our results also show that certain mutations occur at a high frequency in some Brazilian regions and not others. These variations could be due to mutation founder effects, which have been described for other genes in Brazil. These findings should be explored in larger cohorts from specific Brazilian regions to assess whether in these areas, screening with a panel of recurrent mutations would be effective.

Materials and Methods

Laboratory reports of BRCA1 and BRCA2 testing showing pathogenic or likely pathogenic germline mutations were compiled from 28 public and private health care offices located in 11 Brazilian states, including the main reference centers for Genetic Cancer Risk Assessment (GCRA) in Brazil. Not all probands were subjected to a comprehensive BRCA testing (full BRCA sequencing and multiplex ligation-dependent probe amplification, MLPA). The genetic testing was performed using distinct methodologies, including full gene analysis by Sanger or next generation sequencing, point mutation analysis by Sanger or genotyping methods (as HISPANEL), and MLPA for analysis of large genomic rearrangements. Most data came from institutions participating in the Brazilian Hereditary Cancer Network (BHCN), convened by the Brazilian National Cancer Institute (INCA, Instituto Nacional de Cancer) and partially supported by public funding from the National Council for Scientific and Technical Development (CNPq)31. These centers, mostly public hospitals, are established in the Cities/States of Belem/Pará (in the Northern region, encompassing the Amazon basin), Salvador/Bahia (in the Northeastern region), Vitória/Espírito Santo, Rio de Janeiro/Rio de Janeiro, São Paulo/São Paulo, Ribetano Preto/São Paulo, Barretos/Sao Paulo (in the Southeastern region) and Porto Alegre/Rio Grande do Sul (in Southern Brazil). In addition, public or private health care offices from the States of Amazonas (Northern region and the Amazon basin), Minas Gerais (Southeastern region), Rio Grande do Norte, Ceará (Northeastern region) and Santa Catarina (Southern region) also contributed with molecular data from their patients (Fig. 1). All subjects were unrelated and fulfilled HBOC criteria for BRCA testing. Some of the mutations described in this manuscript were also described in prior population/region-specific prevalence studies.12,25,26,31,42–45. This project was approved by the Institutional Review Board from Hospital de Clínicas de Porto Alegre (approval n° 10-0521) and all individuals provided written or verbal consent for BRCA testing. All methods were performed in accordance with the relevant guidelines and regulations, and all data supporting the results are shown in the Supplementary Dataset.

The Human Genome Variation Society (HGVS) nomenclature guidelines (http://www.ncbi.nlm.nih.gov/pubmed) were used to annotate identified variants and the ClinVar database (www.ncbi.nlm.nih.gov/clinvar/) was used to determine the biological significance of all reported variants. For novel variants, BRCA Share (formerly known as UMD, http://www.umd.edu/), LOVD (http://www.lovd.nl/3.0/home), ARUP (http://arup.utah.edu/database/BRCA1) and BRCA Exchange (http://brcaexchange.org/) databases were also checked. Current ACMG guidelines were also used for further classification. BRCA1 and BRCA2 domains were defined using the boundaries in the Pfam database (http://pfam.xfam.org).

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Author Contributions
E.I.P., D.M.C., B.A. and P.A.P. conceived the project. All authors (E.I.P., D.M.C., B.A., M.A.M.M., A.R.S., K.A.S., H.C.R.G., R.M.R., C.P.S., N.P.C., M.I.A., R.C.B., M.N.C.F., F.B.M., F.R.V., A.C.E.S., H.N.S., K.R.L.S., C.B.O.N., C.M.B., J.B., O.A., M.D.P.E.D., T.B.P., A.C.L., R.G., T.C., I.V.D.S., P.B., D.M., S.N., J.H., J.W., P.A.P.) worked on data curation, investigation and methodology. E.I.P., D.M.C., B.A., M.A.M.M., A.R.S., K.A.S. and P.A.P. performed the formal analysis. E.I.P., D.M.C., B.A., M.A.M.M., A.R.S., K.A.S., R.M.R., J.W. and P.A.P. were responsible for funding acquisition. B.A., P.A.P. wrote the original draft, and E.I.P., D.M.C., B.A., M.A.M.M., A.R.S., K.A.S., R.M.R., J.H. and P.A.P. reviewed and edited the manuscript.

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