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VALIDATION OF NCCN CRITERIA FOR GENETIC TESTING IN HBOC SYNDROME IN BRAZIL

Validação dos critérios da NCCN para testagem genética na Síndrome de Câncer de Mama e Ovário Hereditários no Brasil

Andre Vallejo da Silva[1]*, José Claudio Casali da Rocha[2,3], Guilherme Olivetti Guarneri[3]

ABSTRACT

Objective: To identify genetic mutations in BRCA1 and BRCA2 genes in women suspected of HBOC syndrome and to correlate them with NCCN testing criteria to verify its impact on mutation finding rates, as well as to identify the relevant criteria, the frequency and type of found mutations and the relative importance of each NCCN criteria. **Methodology:** A database with all the cases tested for HBOC by the second author from 2010 to 2016 was built, and the variables of interest were annotated and then analyzed with a statistical package to find the relevant variables. **Results:** A total of 171 patients was tested and 38 had deleterious mutations (22%). Criteria with significant association to the present mutations were the total numbers of relatives with cancer (p=0.02) and Ashkenazi lineage (p=0.001). Age of the youngest relative with cancer below 49 was not significant in this sample (p=0.1). There is a strong correlation between mutated patients and NCCN criteria (p=0.0001), but we found no such correlation between the presence of NCCN testing criteria and the presence of mutation (p=0.11). Regarding the use of NCCN criteria to find BRCA mutations, sensitivity was 0.947, specificity was 0.068, PPV was 0.225 and NPP was 0.818. Accuracy was 0.263. **Conclusion:** The incidence of BRCA1 and BRCA2 deleterious mutations in our study was similar to that found in other populations. NCCN criteria were a poor predictor of deleterious mutation in BRCA1 and BRCA2 in general, although most mutant patients had at least one NCCN testing criteria, specially increasing number of affected relatives and Ashkenazi lineage.

KEYWORDS: Gene; breast cancer; genetics; mutations.

RESUMO

Objetivo: Identificar as mutações genéticas nos genes BRCA1 e BRCA2 em mulheres com suspeita de Síndrome de Câncer de Mama e Ovário Hereditários e correlacioná-las com os critérios de testagem da *National Comprehensive Cancer Network* (NCCN), a fim de verificar o seu impacto nas taxas de achados de mutação, bem como identificar os critérios relevantes, a frequência e o tipo de mutações encontradas e a importância relativa de cada critério da NCCN. **Metodologia:** Desenvolveu-se uma base de dados com todos os casos testados para a Síndrome de Câncer de Mama e Ovário Hereditários pelo segundo autor de 2010 a 2016. As variáveis de interesse foram anotadas e, em seguida, analisadas por meio de um pacote estatístico para encontrar variáveis relevantes. **Resultados:** Um total de 171 pacientes foi testado e 38 apresentavam mutações prejudiciais (22%). Os critérios com uma associação significativa às mutações presentes foram os números totais de parentes com câncer (p=0,02) e a descendência Ashkenazi (p=0,001). A idade do parente mais jovem com câncer abaixo de 49 anos não foi significativa nesta amostra (p=0,1). Houve uma forte correlação entre pacientes com mutações e os critérios da NCCN (p=0,0001), mas não encontramos tal correlação entre a presença de testes de NCCN e a presença de mutação (p=0,11). Com relação ao uso dos critérios da NCCN para encontrar mutações BRCA, a sensibilidade foi de 0,947, a especificidade foi de 0,068, PPV foi de 0,225 e NPP foi de 0,818. A acurácia foi de 0,263. **Conclusão:** A incidência de mutações prejudiciais de BRCA1 e BRCA2 em nosso estudo foi semelhante àquela encontrada em outras populações. Os critérios da NCCN foram preditores fracos de mutação prejudicial no BRCA1 e no BRCA2 no geral, embora a maioria dos pacientes mutantes tenha tido, no mínimo, um critério de teste da NCCN, especialmente aumentando o número de parentes afetados e a descendência Ashkenazi.

PALAVRAS-CHAVE: Genes; câncer de mama; genética; mutações.

[1]Universidade Federal Fluminense – Rio de Janeiro (RJ), Brazil.
[2]Hospital Erasto Gaertner – Paraná (PR), Brazil.
[3]School of Medicine, Pontifícia Universidade Católica do Paraná – Paraná (PR), Brazil.
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***Corresponding author:** mastologia@pobox.com
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INTRODUCTION

Statistics from the National Institute of Cancer (INCA) point out the occurrence of 57,960 new cases of breast cancer in Brazil in 2016, which represents around 28% of all female cancers in the country.[1] Among the risk factors for the development of breast cancer, genetic heritage has surely the biggest impact. A risk indication is the history of the disease in mother or sister – the lower the age at diagnosis, with more cases in the family, the higher the risk for the woman. Several genes of susceptibility to breast cancer have already been characterized and mostly of them are of low penetrance, i.e. they slightly modify the risk for cancer development throughout life (<15%), and they are common in the general population. The so-called genes of moderate penetrance have relatively rare alleles and grant the bearer an increase of moderate risk (15 to 30%).

In this research, we discussed the clinical and genetic aspects of the Hereditary Breast and Ovarian Cancer Syndrome (HBOC) caused by a germinative mutation of high penetrance in the genes BRCA1 and BRCA2; then, we correlated it with the criteria established by the National Comprehensive Cancer Network (NCCN) for testing patients with the syndrome. The aim was to identify, in the Brazilian population, the relevant variables which comply with the genetic testing criteria of patients with HBOC and to identify, in the studied population, the genetic mutations and their frequency. Also, to assess the number of occurrences of mutations found for each NCCN criteria, evaluating its resolution.

According to Yiannakopoulou[2], family groups of cancer correspond to 20 to 30% of the breast cancer cases and they are especially caused by genes of medium and low penetrance; only a small part is caused by genes of high penetrance. Of this last group, BRCA mutations respond to about 75% of the cases. In a recent cohort of 9,856 patients with mutations detected in these genes, the cumulative risk of breast cancer until the age of 80 was of 72% for BRCA1 and 69% for BRCA2, whereas the risk of ovarian cancer was of 44% and 17%, respectively. In addition, we found that the penetration varies according to the location of the variants regarding the *locus* in the gene[3].

There are few prevalence studies of mutations in the BRCA genes in the Brazilian population, which has a highly complex and diversified genetic inheritance. Carraro et al.[4], studying 54 patients with breast cancer diagnosed at the age of 35, found a rate of 20.5% of cases with mutations in the BRCA1 (13%) and BRCA2 (7.5%) genes. Gomes et al.[5] found 2.3% of the cases with mutations in BRCA1 and BRCA2 in a non-selected population of 402 patients with breast cancer.

Database construction

We analyzed the medical records, heredograms and exams results from 171 patients' follow-up from the clinic of Dr. José Claudio Casali da Rocha, during the period between January 2012 and April 2016. We extracted the variables seen in Table 1 that are stored in a database developed specifically for this purpose in the software File Maker Pro 13.

Correlation with NCCN criteria

The annotated variables from each case were assessed based on the criteria from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Genetic/Familial High-Risk Assessment: Breast and Ovarian, version 2.2016 (available at NCCN.org), and signalized according to the recommendation created by this

Table 1. Variables analyzed in the study.

Variable	Variable
Identification	Number of relatives, level of family degree, and type of tumor from relatives.
Date of birth	Associated benign pathologies.
Gender	Performed surgeries.
Lineage	Has been through radiotherapy?
Breast cancer?	Has been through chemotherapy?
Age in the first breast cancer	Has used tamoxifen?
Histology	Has used aromatase inhibitor?
Second synchronic breast cancer?	Has used Herceptin?
Second metachronic breast cancer?	
Bilateral breast cancer?	
Receptors of estrogen, progesterone, Ki67 and HER2	
Ashkenazi?	
Presence of other cancers? Which ones?	
Found mutations	

protocol, in the event they obtained or did not obtain the testing criteria, and by the presence or absence of mutations.

Comparison between the local indication and the NCCN indication

For each case recorded in the database, we made a comparison between the presence, or not, of mutations and the NCCN criteria indicating the testing ("NCCN variable").

1. Cases in which these two variables coincided were signalized as "concordant NCCN".
2. Cases in which the testing was positive and the NCCN criterion for testing was not obtained were annotated as "negative NCCN".
3. Cases in which the testing was negative and the NCCN criterion for testing was obtained were annotated as "positive NCCN".

RESULTS

In the sample of 171 sequenced patients, we found 38 women with pathogenic or probably pathogenic variants. The probably pathogenic variants included were considered significant within the genetic counseling context. The NCCN criteria associated with this finding with statistical significance were the total amount of relatives with cancer (p=0.02) and the Ashkenazi lineage (p=0.001).

We did not find a statistically significant association between the presence of the following NCCN criteria and the presence of pathogenic variants: age of the youngest relative with breast cancer below 49 years old (p=0.1), mean of age in the groups (41.2 years old in the group of pathogenic variants against 42 years old in that without these variants, p=0.4) and presence, or not, of NCCN criteria (36 mutated patients with NCCN criteria and another 2 mutated without any criteria, p=0.11).

Table 2 lists the pathogenic or probably pathogenic variants found in 38 patients.

DISCUSSION

We need to develop criteria for indicating proper genetic testing to the Brazilian reality from the scientific point of view, identifying the genetic syndromes and their most frequent associated mutations in Brazil within a context of genetic counseling based on heredogram. Also from the social point of view, to identify the relevance and impact that the identification of these mutations will have on our population, especially in that part covered by the public health. Based on the scarce availability of resources for health actions in the public service (without forgetting the same problem in the supplemental health), we have been introduced to a problem that is analogous to that discussed, for instance, establishment of limits to the mammographic screening. Is it viable, in terms of public health, to extend the annual screening after the 70 years? Does the decrease of treatment morbidity and overall survival obtained through this tracking overcome the investment that is not done in other areas? If we reflect upon the coverage for genetic testing in Brazil, we will powerfully have to make the same choices. Is it worth to test all patients younger than 60 years with triple negative tumors as pointed out by the NCCN criteria, adopted in a society where the economic relations in health are completely different? What will be the impact of this testing in the morbidity and mortality of our patients and their families? In order to achieve the capacity of assessing all of these questions, based on solid scientific evidence, we need to hugely advance in the hereditary cancer epidemiology in our country, which depends on clinical research investment – something that does not seem a possible reality in a close horizon. Clearly, the moment requires a joined assessment of the genetic and mastology societies in a way to establish a consensus on which criteria should we import and support, considering the theoretical knowledge obtained in developed countries and in our socioeconomic reality.

The genetic syndromes of hereditary breast cancer, of which the HBOC is the main representative, have as basic characteristic being dominant autosomal inheritance syndromes. Therefore, we should not forget that the main criterion for diagnosing these syndromes continues to be family history, represented visually by the heredogram which, thus, is still an essential tool for making decisions. The other NCCN criteria (like age and histology) may be considered presumption criteria in the absence of a significant heredogram. We may indicate preventive measurements based only on the heredogram, in the event of no possibility of genetic sequencing, but only within a context of genetic counseling carried out by a properly trained professional. Hence, not only this kind of indication is possible but also the opposite, i.e. the counterindication of aggressive preventive measurements even in the presence of known pathogenic variants. The awareness of this fact has a remarkable social importance, especially in a society that is economically poor as ours, and it allows providing medicine with better quality, even in the absence of technological resources. This discussion, however, should not be considered an approval to aggressive preventive attitudes that are habitual and routinely conducted only with clinical indication. It should also be considered a possible attitude of exception to be adopted by a skilled professional in the absence of genetic sequencing possibility, which is still the gold standard in the determination of known hereditary cancer syndromes.

In order to reflect on the importance of what we have just discussed, consider an analysis of the prevalence table of pathogenic variants in BRCA1 and BRCA2 in non-Ashkenazi women – available at Myriad[6] website, which probably has the greatest accumulation of data in the planet. It is worth noting this company was,a few years ago, the holder of the "patent" of BRCA genes (Prevalence Tables of Deleterious Mutations in BRCA1 and BRCA2 n.d.)[6].

Table 2. Found variants.

Gene/transcribed	Classification	Frequency	Protein	Mollecular consequence	Variation	dbSNP
BRCA1_NM_007294.3	Possibily pathogenic	0.00247%	p.Leu1844Arg	Missense	Single nucleotide variant	rs80357323
BRCA1_NM_007294.3	Pathogenic	413,000%	p.Gln563Ter	Nonsense	Single nucleotide variant	rs80356898
BRCA1_NM_007294.3	Pathogenic	0.1565%	p.Gln1756Profs	Frameshift	Duplication	rs80357906
BRCA1_NM_007294.3	Pathogenic	0.00082%	p.Ser1655Tyrfs	Frameshift	Deletion	rs80359876
BRCA1_NM_007294.3	Pathogenic	0.001%	p.Arg71Gly	Missense	Single nucleotide variant	rs80357382
BRCA1_NM_007294.3	Pathogenic	0.001%	p.Ser1389Terfs	Frameshift	Deletion	rs80357572
BRCA1_NM_007294.3	Pathogenic		p.Ser1655Phe	Missense	Single nucleotide variant	rs80357390
BRCA1_NM_007294.3	Pathogenic	0.024%	p.Glu23Valfs	Frameshift	Deletion	rs386833395
BRCA1_NM_007294.3	Pathogenic		p.Trp1782Ter	Nonsense	Single nucleotide variant	rs80357219
BRCA1_NM_007294.3	Pathogenic		p.Gln1111Asnfs	Frameshift	Deletion	rs80357701
BRCA1_NM_007294.3	Pathogenic			Intron variant	Single nucleotide variant	rs80358050
BRCA1_NM_007294.3	Pathogenic			Splice acceptor variant	Single nucleotide variant	rs80358054
BRCA2_NM_000059.3	Pathogenic	0.001%	p.Met1Arg	Missense	Single nucleotide variant	rs80358547
BRCA2_NM_000059.3	Pathogenic	0.001683%	p.Lys2162Asnfs	Frameshift	Deletion	rs80359598
BRCA2_NM_000059.3	Possibily pathogenic			Splice acceptor variant	Single nucleotide variant	rs397507404
BRCA2_NM_000059.3	Pathogenic	0.002%	p.Ala938Profs	Frameshift	Deletion	rs80359351
BRCA2_NM_000059.3	Benign	0.325%	p.Arg2034Cys	Missense	Single nucleotide variant	rs1799954
BRCA2_NM_000059.3	Pathogenic	0.027%	p.Ser1982Argfs	Frameshift	Deletion	rs80359550
BRCA2_NM_000059.3	Pathogenic		p.Ser2219Ter	Nonsense	Single nucleotide variant	rs80358893
BRCA2_NM_000059.3	Pathogenic	0.002%	p.Ala938Profs	Frameshift	Deletion	rs80359351
BRCA2_NM_000059.3	Pathogenic		p.Asn1603Thrfs	Frameshift	Deletion	rs397507743
BRCA2_NM_000059.3	Pathogenic		p.Tyr3049Ter	Nonsense	Single nucleotide variant	rs886040823
BRCA2_NM_000059.3	Possibily pathogenic	0.00239%		Deleção	Point mutation	rs276174816
BRIP1 NM 032043.2	Pathogenic		p.Trp647Cys	Missense	Point mutation	rs786202760
FANCM NM 020937.3	Possibily pathogenic		p.Leu1950Val	Missense	Point mutation	rs146436929
BRCA2_NM_000059.3	Possibily pathogenic			Missense	Point mutation	rs81002875
BRCA1 Gene complete deletion	Pathogenic				Deletion	
BRCA1 Gene complete deletion	Pathogenic				Deletion	
BRCA2 Undescribed	Possibily pathogenic		p.L2253fs	Frameshift	Deletion	
BRCA2 Undescribed	Possibily pathogenic			Deleção	Point mutation	
BRCA1 Undescribed	Possibily pathogenic			Nonsense	Point mutation	
BRCA1 Undescribed	Possibily pathogenic			Missense	Point mutation	
BRCA2 Undescribed	Possibily pathogenic			Nonsense	Point mutation	
BRCA2 Undescribed	Possibily pathogenic		p.Ser723Leu	Nonsense	Point mutation	
BRCA1 Undescribed	Possibily pathogenic			Missense (splincing)	Point mutation	
BRCA1 Undescribed	Possibily pathogenic			Missense (splincing)	Point mutation	

The only groups with prevalence of pathogenic variants above 40% are those in which there is the presence of ovarian cancer in the proband 's family; in the absence of occurrence (in the patient or in a close relative), the prevalence of pathogenic variants is of 21% at most.

Some limitations in our research should be pointed out, because they affect the level of certainty of the obtained conclusions.

The presence of ovarian cancer was low in the sample and did not allow the relevance of this criterion, which, in more robust samples, is always more important in the assumption of the HBOC presence. Also as a function of the sample size, the presence of two mutated patients without NCCN criterion was responsible for the non-statistical significance of the NCCN criteria presence.

CONCLUSION

The incidence of pathogenic variants in the BRCA1 and BRCA2 genes was similar to that found in other studies. The NCCN criteria were poor predictors of the presence of pathogenic variants in the BRCA1 and BRCA2 genes, although most of the pathogenic covariant patients had at least one NCCN criterion, especially a higher number of relatives with cancer and Ashkenazi ascendency.

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