TP53 Arg72Pro Genetic Polymorphism and Young Women with Breast Cancer: Case-Control Study in Brazil

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Polimorfismo Genético TP53 Arg72Pro e Câncer de Mama em Mulheres Jovens: Estudo Caso-Controle no Brasil

Polimorfismo Genético TP53 Arg72Pro y Cáncer de Mama en Mujeres Jóvenes: Estudio de Casos y Controles en el Brasil

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ABSTRACT

Introduction: Breast cancer is the most common cancer in women and incidence and mortality rates are increasing among young women worldwide, including Brazil. *TP53* Arg72Pro polymorphism (rs1042522) has been associated with breast cancer, due to its important role in cell cycle that impacts the development of cancer. **Objective:** To determine the magnitude of the association between *TP53* Arg72Pro polymorphism and breast cancer development in young Brazilian women. **Method:** Hospital-based case-control study conducted in Rio de Janeiro with 268 confirmed breast cancer cases and 277 controls with women enrolled among hospitalized patients without neoplastic diseases or their companions at three public hospitals. **Results:** The genotype frequency was 46.57% for Arg/Pro, 35.74% for Arg/Arg, and 17.69% for Pro/Pro among healthy controls and 41.04% for Arg/Pro, 46.64% for Arg/Arg, and 12.31% for Pro/Pro among breast cancer cases. The genotypes Pro/Pro (OR=0.46; 95% CI=0.27-0.80, in comparison with Arg/Arg genotype) and Pro allele in dominant model (OR=0.65; 95% CI=0.45-0.92, in comparison with Arg/Arg genotype) were statistically associated with a protective effect for breast cancer among young Brazilian women. Also, family history of breast or ovary cancer (OR=2.18; 95% CI=1.37-3.46) and tobacco use (OR=1.74; 95% CI=1.14-2.68) were statistically associated with breast cancer. **Conclusion:** Further studies are necessary to confirm that Arg72Pro polymorphism can be a protective factor for breast cancer development among young women, since ethnicity can influence genotypes frequencies and the risk of developing breast cancer.

Key words: breast neoplasms; genes, p53; polymorphism, genetic; young adult.

RESUMO

Introdução: O câncer de mama é o mais comum em mulheres e as taxas de incidência e mortalidade estão aumentando entre mulheres jovens em todo o mundo, inclusive no Brasil. O polimorfismo *TP53* Arg72Pro (rs1042522) tem sido associado ao câncer de mama em razão do seu importante papel no ciclo celular que pode impactar o desenvolvimento do câncer. **Objetivo:** Determinar a magnitude da associação entre o polimorfismo *TP53* Arg72Pro e o desenvolvimento de câncer de mama em mulheres jovens brasileiras. **Método:** Estudo caso-controle de base hospitalar realizado no Rio de Janeiro com 268 casos confirmados de câncer de mama e 277 controles com mulheres cadastradas entre pacientes internados sem doenças neoplásicas ou seus acompanhantes em três hospitais públicos. **Resultados:** A frequência genotípica foi de 46,57% para Arg/Pro, 35,74% para Arg/Arg e 17,69% para Pro/Pro entre controles saudáveis e 41,04% para Arg/Pro, 46,64% para Arg/Arg e 12,31% para Pro /Pro entre os casos de câncer de mama. Os genótipos Pro/Pro (OR=0,46; IC 95%=0,27-0,80, em comparação ao genótipo Arg/Arg) e o alelo Pro no modelo dominante (OR=0,65; IC 95%=0,45-0,92, em comparação com o genótipo Arg/Arg) foram estatisticamente associados a um efeito protetor para o câncer de mama em mulheres jovens brasileiras. Além disso, história familiar de câncer de mama ou ovário (OR=2,18; IC 95%=1,37-3,46) e tabagismo (OR=1,74; IC 95%=1,14-2,68) foi estatisticamente associada ao câncer de mama. **Conclusão:** Novos estudos são necessários para confirmar que o polimorfismo Arg72Pro pode ser um fator de proteção para o desenvolvimento de câncer de mama em mulheres jovens, uma vez que a etnia pode influenciar tanto as frequências desses genótipos quanto o risco de desenvolver câncer de mama.

Palavras-chave: neoplasias da mama; genes p53; polimorfismo genético; adulto jovem.

RESUMEN

Introducción: El cáncer de mama es el cáncer más común en la mujer y las tasas de incidencia y mortalidad están aumentando entre las mujeres jóvenes en todo el mundo, incluido Brasil. El polimorfismo *TP53* Arg72Pro (rs1042522) se ha asociado con el cáncer de mama, debido a su importante papel en el ciclo celular que puede afectar el desarrollo del cáncer. **Objetivo:** Determinar la magnitud de la asociación entre el polimorfismo *TP53* Arg72Pro y el desarrollo de cáncer de mama en mujeres jóvenes brasileñas. **Método:** Estudio de casos y controles de base hospitalaria realizado en Río de Janeiro con 268 casos confirmados de cáncer de mama y 277 controles con mujeres inscritas entre pacientes hospitalizadas sin enfermedades neoplásicas o sus acompañantes en tres hospitales públicos. **Resultados:** La frecuencia de genotipos fue del 46,57% para Arg/Pro, 35,74% para Arg/Arg y 17,69% para Pro/Pro entre controles sanos y 41,04% para Arg/Pro, 46,64% para Arg/Arg y 12,31% para Pro/Pro entre los casos de cáncer de mama. El genotipo Pro/Pro (OR=0,46; IC 95%=0,27-0,80, en comparación con el genotipo Arg/Arg) y el alelo Pro en el modelo dominante (OR=0,65; IC del 95 %=0,45-0,92, en comparación con el genotipo Arg/Arg) se asociaron estadísticamente con un efecto protector frente el cáncer de mama entre mujeres jóvenes brasileñas. Además, los antecedentes familiares de cáncer de mama o de ovario (OR=2,18; IC 95%=1,37-3,46) y el hábito del tabaquismo (OR=1,74; IC 95%=1,14-2,68) se asociaron estadísticamente con el cáncer de mama. **Conclusión**: Son necesarios nuevos estudios para confirmar que el polimorfismo Arg72Pro puede ser un factor de protección para el desarrollo del cáncer de mama en mujeres jóvenes, ya que la etnia puede influir r tanto en las frecuencias de estos genotipos como en el riesgo de desarrollar cáncer de mama.

Palabras clave: neoplasias de la mama; genes p53; polimorfismo genético; adulto joven.

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INTRODUCTION

Breast cancer is the most common among women worldwide[1]. According to the International Agency for Research on Cancer (IARC)[1], 215 million new cases are estimated for 2025 and 769 thousand deaths are expected globally, a clear aggravation of this worldwide public health problem. Furthermore, there has been an increase of incidence and mortality rates by this neoplasm worldwide among young women[2-5].

Literature shows some risk factors as possibly associated with breast cancer development among young women as alcohol and tobacco use, family history of breast cancer and some punctual mutations mainly in *BRCA 1*, *BRCA 2* and *TP53* genes[2,6-8]. *TP53* gene is a constitutive tumor suppressor gene that is part of the biological mechanisms that act in cell control, which encodes a protein called p53[9,10]. This protein is a transcriptional regulator induced by DNA damage, a fact that results in cell cycle arrest with consequent activation of repair mechanisms or even induction of apoptosis[10-13]. Besides mutations, *TP53* is a polymorphic gene and Arg72Pro polymorphism (rs1042522) is the most investigated in relation to associations with different neoplasms, however the results are still conflicting in relation to breast cancer. Presence of this single nucleotide polymorphism (SNP) leads to the encoding of a protein with the amino acid proline (Pro) in codon 72, replacing the amino acid arginine (Arg) encoded by the wild type allele. This substitution is produced by a single nucleotide exchange from guanine to cytosine and can impact in different ways in DNA damage induction according to the presence of Arg or Pro allele[9,10,13]. In brief, Arg protein was reported to be more efficient in inducing apoptosis than the Pro variant, due to the greater efficiency of the Arg variant to localize to mitochondria[10-13]. The allele frequency of Arg in this codon is approximately 70% for the Caucasian population[14].

Given the importance of this gene in the process of cell growth and cancer development, this study aims to evaluate the association between Arg72Pro polymorphism with breast cancer development in young Brazilian women.

METHOD

The study design consisted in a case-control study with women living in the Metropolitan Region of Rio de Janeiro, Brazil and detailed in a previous study[15]. The cases comprehended 268 women with confirmed histopathological diagnosis of breast cancer (ICD 10 50.0-50.9), at the age range of 18-35 years, referred to the National Cancer Institute (INCA), an oncological reference center in the city of Rio de Janeiro, between 1999 and 2009.

The controls included 277 women enrolled among hospitalized patients without neoplastic diseases or patient's companions at three public hospitals, *Hospital Pro-Matre, Instituto Nacional de Traumatologia e Ortopedia Jamil Haddad* (INTO*)* and *Hospital da Lagoa* that offered cost-free care in the same city. Participants signed the informed consent form and were interviewed in-person by skilled interviewers, with the application of a study-designed standard questionnaire. After, peripheral blood samples were collected in EDTA Vacutainer tubes for genomic DNA extraction, following a salting out technique standard protocol[16]. The Institutional Review Board of INCA, *Pro-Matre*, *Hospital da Lagoa* and *Escola Nacional de Saúde Pública* (Ensp/Fiocruz) approved the study (CAAE: 0191.0.031.000-10).

TP53 genetic polymorphism was assessed by previously described polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) protocols with minor modifications[17]. Target DNA amplification was achieved by PCR optimized conditions: a final reaction volume of 25 µL composed of 100-200 ng of DNA, 0.2 mM of each dTNP (Invitrogen), 3 mM of $MgCl_2$, 0.75 U of Platinum Taq DNA polymerase (Invitrogen), 1× PCR buffer (Invitrogen), and 10 pmol of each primer (forward 5'atctacagtccccttgccg3' and reverse 5'gcaactgaccgtgcaagtca3'). The reaction conditions used were a pre-denaturation at 94ºC for 5 min followed by 35 cycles with three steps each (94ºC for 30 s, 68ºC for 30 s, and 72ºC for 40 s), and a cycle of 7 min at 72ºC. Negative controls were included in every run, and amplification success was confirmed in agarose 1.5% gels, stained with Gel Red (Biotium), and visualized under ultraviolet light. Endonuclease digestions were performed in a final reaction volume of 20 µL consisting in 3µL of PCR products, 6U of *BstUI* enzyme (New England Biolabs), and 1× reaction buffer (New England Biolabs), using overnight 60ºC incubation conditions. Genotypes determination was performed in agarose 3% gels.

Genotype distribution goodness-of-fit to Hardy-Weinberg equilibrium was ascertained for controls, using R 2.15.2 software.

Continuous variables were expressed as means ± standard deviation (SD) and differences between them were analyzed using the Mann-Whitney U test. Categorical variables were expressed as percentages and Pearson chi-square was used to analyze differences between them.

Unconditional logistic regression models were used to calculate unadjusted and adjusted odds ratios (OR)

and 95% confidence intervals (95% CI) for association between *TP53* polymorphism and breast cancer, using STATA 10.0 software. P-value <0.05 was used to ascertain occurrence of statistical significance. All confounders (age, skin color, education, pregnancy, age at menarche, hormonal contraceptives use and family history of breast and/or ovary cancer of first-degree relatives) was tested in logistic regression, and those that do not modify breast cancer association and genetic polymorphisms were eliminated at the final model.

RESULTS

Breast cancer cases and controls distribution according to age, skin color, occurrence of pregnancy, age at menarche and family history of breast or ovary cancer are presented at Table 1. Mean age was 31.5 years (±3.4) among cases and 29.9 years (±4.5) among controls. White individuals accounted for 30.2% of the cases and 31.8% of controls; 69.8% of the cases and 68.2% of controls were non-White (p=0.70). Family histories of breast or ovary cancer, in first degree relatives, were reported by 22.8% of the cases and 11.9% of controls and were statistically associated with breast cancer development among young women (OR=2.18; 95% CI=1.37-3.46). Tobacco use also showed statistical association with breast cancer in this population (OR=1.74; 95% CI=1.14-2.68).

The association between Arg72Pro *TP53* polymorphism and breast cancer is presented in Table 2. The genotypes Pro/Pro and Arg/Pro were statistically associated with a protective effect for breast cancer in young women (OR=0.53, 95% CI=0.32-0.89). The OR adjustment for age and skin color revealed a negative association even greater for Pro/Pro genotype (OR=0.46, 95% CI=0.27-0.80), however for Arg/Pro genotype the association becomes non-significant after this adjustment (OR=0.70, 95% CI=0.48-1.02). Considering the dominant model, it was noticed a statistically significant protective effect of the presence of at least one Pro allele (Pro/Pro + Arg/Pro) in comparison to Arg/Arg genotype (OR=0.64; 95% CI=0.45-0.90). The OR adjustment for age and skin color keeps the association (OR=0.65, 95% CI=0.45-0.90).

DISCUSSION

To the best of the existing knowledge, this was the first study to investigate Arg72Pro SNP genotypes frequencies among young women with a histopathological confirmed diagnosis of breast cancer (ICD 10 50.0-50.9) in Brazil. Thus, Arg/Arg genotype was the most frequent in this population (46.64%) with breast cancer, followed by Arg/Pro genotype (41.04%), and Pro/Pro genotype (12.31%). Among cancer cases, it was shown that the frequency of Arg/Arg genotype varied from 8.0% in Brazil to 91.9% in China, while the frequency of the Pro/Pro genotype ranged from 0% in China to 54.0% in Russia[18-20].

The frequency of the Arg/Pro genotypes varied from 8.1% in China to 69.4% in Saudi Arabia[19,21]. In Brazil, for women diagnosed with breast cancer, without age limitation, case-control studies estimated genotypes

Table 1. Distribution of breast cancer cases (n=268) and controls (n=277) according to epidemiological data. Rio de Janeiro, Brazil, 1999-2012

Variables		Cases N (%)	Controls N (%)	OR (95% CI)	P value
Age (yr.)	18-23	7 (2.61)	32 (11.55)	1.00	
	24-29	59 (22.01)	67 (24.19)	**4.03 (1.65-9.80)**	
	30-35	202 (75.37)	178 (64.26)	**5.19 (2.24-12.04)**	0.000[a]
	Mean [SD]	31.49 [3.36]	29.88 [4.43]	**1.11 (1.06-1.16)**	0.000[b]
Skin color	White	81 (30.22)	88 (31.77)	1.00	
	Non-White	187 (69.78)	189 (68.23)	1.08 (0.75-1.55)	0.697[a]
Family history of breast or ovary cancer	No	207 (77.24)	244 (88.09)	1.00	
	Yes	61 (22.76)	33 (11.91)	**2.18 (1.37-3.46)**	0.001[a]
Smoking	No smoker	203 (75.75)	234 (84.48)	1.00	
	Smoker	65 (24.25)	43 (15.52)	**1.74 (1.14-2.68)**	0.011[a]

Captions: OR = odds ratios; CI = 95% confidence interval.
(a) $\chi 2$ test.
(b) Mann-Whitney U test.

Table 2. Distribution of breast cancer cases and controls according to *TP53* genotypes. Rio de Janeiro, Brazil, 1999-2012

TP53* genotypes	**Controls N (%)**	**Cases N (%)**	**Crude OR (95% CI)**	**Adjusted OR[a] (95% CI)**
Arg/Arg	99 (35.74)	125 (46.64)	1.00	1.00
Arg/Pro	129 (46.57)	110 (41.04)	**0.68 (0.47-0.97)**	0.70 (0.48-1.02)
Pro/Pro	49 (17.69)	33 (12.31)	**0.53 (0.32-0.89)**	**0.46 (0.27-0.80)**
Recessive model				
Pro/Pro	49 (17.69)	33 (12.31)	1.00	1.00
Arg/Arg + Arg/Pro	228 (82.31)	235 (87.69)	1.53 (0.95-2.47)	1.62 (0.99-2.64)
Dominant model				
Arg/Arg	99 (35.74)	125 (46.64)	1.00	1.00
Pro/Pro + Arg/Pro	178 (64.26)	143 (53.36)	**0.64 (0.45-0.90)**	**0.65 (0.45-0.92)**

Captions: OR = odds ratios; CI = 95% confidence interval.
(*) TP53 Hardy-Weinberg p=0.72.
(a) Age and skin color adjusted.

frequency ranged from 8.0% to 55.5% for Arg/Arg genotype, from 40.3% to 60.0% for Arg/Pro genotype, and from 4.2% to 32.0% for Pro/Pro genotype[22-27]. There is also an ethnicity variation among breast cancer women, according to the Brazilian regions, with Arg/Arg genotype frequencies ranging from 44.7% to 55.5% in Brazil's South region[23,24,26]; whereas in the Southeast and Northeast regions, Arg/Pro genotype was the most frequent, varying from 41.4% to 60%[22,25,27].

This wide variation of Arg72Pro SNP frequencies according to ethnicity, favors the observation of different associations of this SNP with the development of breast cancer[28]. Among the young women investigated, the genotype Pro/Pro was statistically associated with a protective effect for breast cancer (OR=0.46, 95% CI=0.27-0.80, Arg/Arg genotype as reference and adjusted for age and skin color). Alawadi et al.[21] in their case-control study in Saudi Arabia with 288 breast cancer women and 188 controls also found that Pro/Pro genotype was a protective factor for breast cancer (OR=0.17, 95% CI=0.07-0.41), with a median age of 54.74 years to cases and 48.74 years to controls and the genotyping was performed by the PCR-RFLP method[21].

Other three case-control studies[29-31] also corroborate the conclusions that Pro/Pro genotype is a protective factor for breast cancer development, although in these articles the OR was calculated using Pro/Pro genotype as reference. So, Yulug et al.[29], studying Turkish and Greek populations with 138 breast cancer cases and 138 blood donors as controls in Greece, and 274 breast cancer cases and 221 blood donors as controls in Turkey, concluded, among Turkish women, a great statistical association of the Arg/Arg genotype with breast cancer (OR=2.16, 95% CI=1.08-4.31)[29]. For Greek women, a great association, but without statistical significance with Arg/Arg genotype was found (OR=7.93, 95% CI=0.95-65.98). Genotyping was also performed by the PCR-RFLP method and median age was 49.30 years to cases and 46.59 years to controls[29]. Gochhait et al.[30], in their case-control study conducted in India with 243 breast cancer women and 333 healthy controls, observed, by sequencing method, a great statistical significance association of breast cancer development with Arg/Arg genotype (OR=2.30, 95% CI=1.4-3.6)[30]. And Proestling et al.[31], in Austria, with 267 breast cancer women and 220 healthy controls, with global median age of 58.7 years, noticed a great statistical significance association of this neoplasia with Arg/Arg genotype (OR=2.38, 95% CI=1.01-5.93).

Further, the dominant model (Pro/Pro+Arg/Pro *versus* Arg/Arg) suggested a significant protective effect of the presence of Pro allele for breast cancer, among Brazilian young women (OR=0.65; 95% CI=0.45-0.92). Similar result was observed by Liu et al.[19], who conducted a case-control study in China with 1,100 breast cancer women and 1,400 controls paired by age, finding a protective association of Pro/Pro+Arg/Pro *versus* Arg/Arg (OR=0.45, 95% CI=0.35-0.59).

Although the studies referenced corroborate the present results, many studies do not find an association between the presence of this polymorphism and the development of breast cancer[22]. Apparently, most of the studies that found a statistically significant association suggest that the presence of Pro allele is a risk factor for the development of the disease and not a protective instead[24,32-34]. Unfortunately, most of these studies were very small samples-based, a clear limitation, except for The

Breast Cancer Association Consortium[35], which combines the population of different countries.

Indeed, this combination of different populations can also be a limitation, considering the wide variation in Arg72Pro SNP frequencies, according to ethnicity, that can influence its association with breast cancer. This was detected in two recent metanalysis about the theme. Gonçalves et al.[36] showed a small increased risk due to the presence of Pro allele in the dominant model (OR=1.11, 95% CI=1.02-1.21; *versus* Arg/Arg), but not in Asia, where the risk was associated with the presence of Arg allele (OR=1.23, 95% CI=1.07-1.41; *versus* Pro/Pro - Recessive model). Diakite et al.[37] found that Pro allele was associated with an extremely small increased risk of breast cancer in the dominant model for overall analyses (OR=1.09, 95% CI=1.02-1.16). This result was quite similar for Caucasian populations, but the authors did not find statistically significant results in the Asian population[37]. So, despite the importance of *TP53* gene, the association of Arg72Pro SNP and the development of breast cancer remains inconclusive.

The study of Arg72Pro SNP is complex since each one of the alleles can promote different BRAC1/2 transcription, causing dissimilar advantages in terms of protecting cells against breast tumorigenesis[30,38,39,40-45]. Pro allele appears to better perform G1 arrest than the Arg variant protein[30,46,47]. Besides that, Pro allele shows a decreased efficiency at triggering apoptosis, mainly due to its decreased ubiquination by MDM2 and to its increased efficiency to bind apoptosis-stimulating inhibitor of apoptosis-stimulating p53 protein (iASPP)[30,41,48-52]. Furthermore, in order to understand cancer as a multifactorial disease, it is also important to know the population's ethnicity and age for breast cancer development, other mutations or polymorphisms, especially those in the BRCA1 or BRCA2 genes, as well as environment interactions.

Other articles[2,53-57] which investigated the association between Arg72Pro SNP and the development of breast cancer did not limit the age of the study population analyzed or performed subgroup analysis according to this variable, an important aspect to highlight. The combination of pre-and post-menopausal women may not be a good strategy, since some risk factors seem to be different for these two populations[2,53-57]. Besides that, multiple studies[6,58,59] have suggested that breast cancer in young women could be more aggressive, and with worse prognosis, regardless of pathologic variables. The scientific community is starting to consider that breast cancer among young women could present a different biologic entity[58]. In this scenario, it is difficult to compare the present results of a negative association of Arg72Pro *TP53* Pro allele with breast cancer in young women according with the studies published so far.

The main strength of this article is the restriction to young women, which is a form to prevent age from being a confounding factor of the results. Finally, another advantage is the use of a large sample size in comparison with others case-controls studies. However, the genotyping method applied in the present study can be considered a limitation, efforts were endeavored to make sure that PCR-RFLP technique related errors were eliminated through replication analysis for 10% of the samples, in order to validate the correct classification.

It is known that breast cancer is considered a multifactorial disease, suggesting that a single polymorphism is probably insufficient to produce disease phenotype, being necessary environmental factors interacting with gene polymorphism/mutations to affect breast cancer risk[60]. However, biological mechanisms by which such interactions modulate breast cancer risk development among young women is still not totally clear, it is already known that family history of breast or ovary cancer is an important risk factor and it seems that tobacco use may be an important environmental factor associated with breast cancer in this young population[61-66]. In the present study, an association of family history of breast or ovary cancer and tobacco use with breast cancer in young women was found.

Knowledge about family history is considered essential when evaluating young women with breast cancer[67]. Many studies[6,54,55,67] concluded that breast or ovary cancer family history could help to identify individuals at elevated risk for hereditary breast cancer or women who would benefit from increased breast cancer surveillance. Many international guidelines also recommend assessment of family history and screening patients at increased risk of breast cancer[67]. One of them, the American College of Obstetricians and Gynecologists[53], states that screening should include at least a personal cancer history of first and second-degree relatives' cancer history, including information as description of primary cancer type, age of onset, and lineage of the family member.

Several studies[68,69] suggest that tobacco acts since the initiation to neoplastic progression, mainly in cells of epithelial origin, and there are strong evidences that the breast tissue is a target for these carcinogenic effects. Epidemiological studies[70,71] also corroborate these evidences, as tobacco use has been associated with increased risk of breast cancer.

Some studies[72,73] show that breast cancer mortality rate among Brazilian young women has been increasing in all country regions, mostly for women from 30-39 years old and tobacco use is increasing among Brazilian women.

It is quite clear that to investigate the association between tobacco use and the development of breast cancer in young women is an important initiative and bring awareness to these women about all the risks associated.

CONCLUSION

The results suggest that the presence of at least one Pro allele can be a protective factor for breast cancer development among young women. In addition, it is important to cite that the present study is relevant to show Arg72Pro polymorphism frequencies in a mixed-race population such as Brazil, since ethnicity can influence genotypes frequencies and the risk of developing breast cancer in young women. Besides that, future studies with different study designs, genotyping techniques, and larger sample size are required to test hypotheses raised from this investigation.

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CONTRIBUTIONS

The authors participated in all the phases of the manuscript and approved the final version to be published.

DECLARATION OF CONFLICT OF INTERESTS

There is no conflict of interest to declare.

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