**Cancer risk factors in Southern Brazil: Report of a large, matched case-control study**

Juliana Giacomazzi PhD[1,2], Patricia Klarmann Ziegelmann PhD[2,3], Fernando Mariano Obst MD[1,4,5,6], Samanta da Costa RN[1], Camila Matzembacher Bittar PhD[1], Clevia Rosset PhD[7,8], Gabriel Macedo de Souza PhD[7], Hugo Bock PhD[8], Thais Canal BSc[1], Mari Ines Paese[1], Jean Lucas Benvenuti[1], Maria Carolina Buj[1], Patricia Ashton-Prolla PhD[7,9], José Roberto Goldim PhD[10,11] and Roberta Pozza PhD[1,4]

1. Instituto Tacchini de Pesquisa em Saúde/Hospital Tacchini, Tacchini Sistema de Saúde; 2. Programa de Pós-graduação em Epidemiologia, Universidade Federal do Rio Grande do Sul, UFRGS; 3. Departamento de Estatística, UFRGS; 4. Instituto do Câncer, Hospital Tacchini, Tacchini Sistema de Saúde; 5. Oncoclínicas Porto Alegre; 6. Hospital São Lucas da Pontifícia Universidade Católica de Porto Alegre, PUCRS; 7. Laboratório de Medicina Genômica, Hospital de Clínicas de Porto Alegre; 8. Unidade de Pesquisa Laboratorial, Hospital de Clínicas de Porto Alegre; 9. Departamento de Genética, UFRGS; 10. Serviço de Bioética, Hospital de Clínicas de Porto Alegre; 11. Faculdade de Medicina, PUCRS.
Correspondence: Juliana Giacomazzi, Instituto Tacchini de Pesquisa em Saúde/Hospital Tacchini, Tacchini Sistema de Saúde, General Osorio, 235 Bento Gonçalves RS 95700-084, Brazil juliana.giacomazzi@tacchini.com.br

**ABSTRACT**

**Background:** The incidence of cancer is increasing in developing countries like Brazil. The presence of multiple risk factors with varied risk estimates in different studies, and the lack of knowledge about the burden of heredity on cancer makes it even more difficult to design specific prevention programs.

**Objectives:** This study aimed to identify factors associated with cancer by matching cases and controls by age group and sex, and to analyze a multigene hereditary panel testing (MGPT, 26 genes) to breast and colorectal cancer cases (CCR) diagnosed in patients under 50 years of age in Southern Brazil.

**Methods:** A single center, matched case-control study was conducted from March 2018 to March 2021 in a regional cancer center. The cases were comprised of the most prevalent cancers diagnosed and the control group was comprised of individuals without cancer from the same region. Data on socio-demographic characteristics, exposure to cancer risk factors and family history of cancer (FHC) were collected. The MGPT was performed using Illumina Next Generation Sequencing technology. Conditional logistic regression analysis was performed.

**Results:** A total of 1007 cases and 1007 controls were included. Among these, 311 breast, 147 CCR, 132 prostate and 89 lung cancer patients were recruited. MGPT identified pathogenic/likely pathogenic mutations in 24 (32%) women with breast cancer, and in three (18%) women and four (24%) men diagnosed with colorectal cancers. Associations of several risk factors with breast, CCR, prostate, and lung cancers were confirmed in the study.

**Discussion:** A better understanding of population specific risk factors can inform more effective prevention strategies and build on sustainable data for the development of cancer prevention strategies. These efforts in countries where cancer is considered one of the main public health problems also increases the commitment to early detection and surveillance, allowing for more focused and preventive health education.

## INTRODUCTION

Cancer ranks as a leading cause of death and an important barrier to increasing life expectancy in every country in the world. Its incidence is increasing in developing countries, such as Brazil, in which the curves of incidence are rising but vary widely according to geographical region; with the South and Southeast showing the highest rates.[1] This variation may be due to different reasons, especially heterogeneous prevalence and distribution of the main cancer risk factors, several of which are associated with socioeconomic development, but could be due to the heterogeneous genetic makeup of the population in different Brazilian regions (Instituto Nacional do Câncer 2021). Cancer has a complex and multifactorial etiology with a strong interplay between genetic, demographic, hormonal, and environmental factors covering a broad range of conditions, such as age, family history, hormonal history, diet and exercise, body mass index, smoking and alcohol use, and exposure to chemical agents and pesticides.[2]

Better understanding of the of the local/regional risk factors may inform more effective prevention strategies. When risk factors are identified and well understood, healthcare providers can supply individuals with more accurate information on their disease risks and develop tailored risk reducing strategies. These efforts in countries where cancer is considered one of the main public health care problems and, as in Brazil, where 70–80% of the population relies on the public health care system, also increases the commitment of health providers and patients to early detection, allowing for more focused and preventive health education and management.[2,3]

A few studies have been conducted in Brazil to screen for potential risk factors for cancer, with small sample sizes.[4-7] Several Brazilian studies analyzed the prevalence of hereditary phenotypes or genes associated with hereditary predisposition especially among individuals diagnosed with cancer and with a family history for the disease; or evaluated specific founder mutations.[8-12]

Currently, national monitoring data on risk factors among the Brazilian general population are limited. In Southern Brazil there is no comprehensive study evaluating cancer risk factors, nor including germline MGPT in patients diagnosed with cancer under age 50 years.

In this study, we sought to identify genetic, demographic, hormonal and environmental risk factors for high incidence cancers using a large sample of Southern Brazilian individuals. Additionally, we performed germline MGPT in patients diagnosed with the most incident tumors (breast and colorectal cancers) under age 50 years.

## METHODS

### Study Design and Setting

This single-center, hospital-based, matched case-control study was conducted between March 2018 and March 2021 at the Hospital Tacchini, Bento Gonçalves, Rio Grande do Sul, Brazil. This institution is a regional cancer reference center, considered a UNACON (High Complexity Unit in Oncology) for the Northeast region of the Southernmost State of Brazil, Rio Grande do Sul.

### Participants

A case was defined as any individual diagnosed with an invasive cancer receiving treatment in the institution and was invited to participate consecutively. A control was an individual without a cancer diagnosis, matched by age and sex to a case. To recruit controls, invitations to participate in the study were made through social networks. In addition, controls were recruited from a variety of settings, including companies from various sectors in the region and community events. All participants signed a written informed consent before recruitment during a structured face-to-face interview conducted by the Tacchini Research Institute team.

**Variables and Data Collection**

Information on demographic characteristics, cancer risk factors, and family history of cancer were obtained in interviews and through chart review. For cases, the interview occurred during the individual's visits to the hospital for treatment. For controls, it was held in a specific event area reserved for the research team to contact and interview participants

**Molecular analysis**

*Gene selection***:** MGPT was performed with Next Generation Sequencing (NGS) of 26 hereditary cancer predisposition genes using the Hereditary Cancer MASTR panel (Agilent). Genes were selected based on their association with hereditary predisposition for breast and colorectal cancers.

*DNA isolation***:** Genomic DNA was extracted from peripheral blood leukocytes using the FlexiGene DNA Kit (QIAGEN) and was quantified using the NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific).

*Library preparation for NGS analysis:* libraries were prepared according to the Hereditary Cancer MASTR guide (Agilent Technologies).

*Amplicon-based gene panel protocol***:** Amplification of the entire coding region including the intron-exon boundaries of the genes *BARD1, BRCA1, BRCA2, BRIP1, RAD51C, RAD51D, TP53, MRE11A, RAD50, NBN, FAM175A, ATM, PALB2, STK11, MEN1, PTEN, CDH1, MUTYH, CHEK2, BLM, XRCC2, EPCAM, MLH1, MSH6, PMS2* and *MSH2* was carried out using the BRCA Hereditary Cancer MASTR™ Plus assay kit (Agilent) according to the manufacturer's instructions.

*Sequencing*: Products were subsequently analyzed by NGS using the Illumina platform, MiSeq (Illumina, San Diego, California, United States), using v3 sequencing kit (600-cycle), 5% PhiX control and read depth of at least 30x per base. The results were analyzed using the MASTR reporter data analysis software, with parameters optimized for reliable variant calling, including copy number variation detection. Variants were classified in five categories according to the ACMG (American College of Medical Genetics) guideline (Richards et al. 2015).[13] A list of the genes analyzed by this hereditary cancer panel and their association with breast and colorectal cancer and syndromes is presented in Supplementary Table 1.

**Ethical Considerations**

This study was approved by the Research Ethics Committee of Hospital Tacchini (CAAE number 85223818.1.1001.5305).

**Statistical Analysis**

Conditional logistic regression analysis was performed to evaluate associations between various risk factors and cancer, measured by odds ratios with 95% confidence intervals. First, all types

of cancer were included in the same model; thereafter, each model was adjusted by each cancer type (prostate, lung, colorectal and breast). To eliminate possible confounding effects all results were adjusted by educational level. For the multivariable analysis, all risk factors with p*<0.20* on bivariate analysis were included and only the ones with $p < 0.05$ were kept in the final models. All statistical analyses were performed using the Survival R Package.[14]

**Role of the funding source**

The study sponsor was Tacchini Sistema de Saúde, through a donation for this research project. The study sponsor was not involved in study design, data collection, analysis, interpretation of data, writing, or in the decision to submit the paper for publication.

**RESULTS**

A total of 1007 cancer cases and 1007 controls were included in this study. Overall, cases and controls were residents of the Northeastern region of Rio Grande do Sul (Brazil).

Fifty-five percent were women, 78.9% were over the age of 50 years at recruitment and most were married. There was no difference between the groups regarding ethnicity; most were white. Cancer patients had less education compared to controls. Because of this, education was included in the statistical model as an adjustment factor. The socio-demographic characteristics of the individuals included are described in Table 1. Of the cases, 311 were diagnosed with breast cancer, 147 with colorectal cancer, 132 with prostate cancer, and 89 with lung cancer (Table 2).

Overall risk factors included first or second-degree family history of cancer, tobacco consumption, alcohol consumption, pesticide exposure, solvent/glue exposure, and body mass index, which were more frequently and significantly (p<0.20) associated with cancer cases in a bivariate analysis. Physical activity was not associated with cancer in this series (OR=0.9; CI%:0.7-1.1; p=0.28). In the multivariate analysis, a first or second-degree family history of cancer (OR=6.1; CI: 4.7-7.9; p<0.001), tobacco consumption (OR=8.8; CI: 6.2-12.5; p<0.001), alcohol consumption (OR=9.0; CI: 4.3-19.1); p<0.001), pesticide exposure (OR=2.9; CI: 1.9-4.4; p<0.001), solvent/glue exposure (OR=1.9; CI: 1.0-4.3; p=0.04) and body mass index (BMI) < 24 (OR=1.5; CI: 1.1-.2.1; p=0.009) were independently associated with cancer.

The frequency of use according to pesticide class was similar between cases and controls, demonstrating similar behavior per chemical class usage between groups, although the frequency of use was higher in the cancer group (Supplementary Table 2).

**Breast cancer**

All risk factors described in Table 3, except physical activity and contraceptive use, were significantly (p<0.20) associated with breast cancer cases in bivariate analysis. In a multivariate analysis, a first or second-degree family history of cancer ($OR_a$=6.2; 95% CI: 4.1-9.5; p<0.001); tobacco consumption (ORa=4.2 (2.4-7.5), p<0.001); and hormone replacement therapy use (ORa=3.0, CI: 1.2-7.6; p=0.02) were independently associated with a higher risk of breast cancer.

**Colorectal cancer**

All risk factors described in Table 3, except solvent/glue exposure and physical activity were significatively associated with colorectal cancer in bivariate analyses. In a multivariate analysis, a first or second-degree family history of cancer (ORa=4.7, CI: 2.8-8.6; p<0.001); tobacco

consumption (ORa=3.1; CI: 1.5-6.3; p=0.002) and BMI < 24 (ORa=2.1; CI: 1.0-4.3; p=0.04) were independently associated with a higher risk of colorectal cancer.

**Prostate cancer**

All risk factors described in Table 3, except physical activity and BMI, were significatively associated with prostate cancer in a bivariate analysis. A first or second-degree family history of cancer (ORa 6.7; CI: 2.8-15.5; p<0.001); tobacco consumption (ORa=10.5; CI: 4.2-26.3; p<0.001) and alcohol consumption (ORa=7.3; CI: 1.3-40.5; p=0.01) were independently associated with a higher risk of prostate cancer.

**Lung cancer**

All risk factors described in Table 3 were significatively associated with lung cancer in a bivariate analysis. In multivariate analysis, a first or second-degree family history of cancer (ORa=30.2, CI: 4.2-218.0; p<0.001); tobacco consumption (ORa=1331.9; CI: 48.1-36884.9; p=0.002) and BMI < 24 (ORa=9.3; CI: 1.3-67.8; p=0.02) were independently associated with a higher risk of lung cancer. Physical activity conferred risk reduction (ORa=0.07; CI: 0.01-0.54; p=0.009).

Germline multigene panel testing of 26 cancer predisposition genes identified pathogenic/likely pathogenic variants in 24 (32%) women with breast cancer, and in three (18%) women and four (24%) men diagnosed with colorectal cancers, while at least one variant of uncertain significance (VUS) was identified in 20 (27%) women and one (50%) man diagnosed with breast cancer, and in four (24%) women and three (18%) men diagnosed with colorectal cancers. Among breast cancer and colorectal cancer cases tested, 98.7% and 97.0% met at least one criterion for hereditary cancer predisposition syndromes (Table 4). The detailed molecular findings are presented in Table 5.

**DISCUSSION**

The present study confirmed the association of several factors associated with breast, colorectal, prostate, and lung cancers. Overall, a first or second-degree family history, a family history of cancer in patients under age 50 years, and tobacco consumption were associated with cancer. Pre-menopausal status, abortion, and hormone replacement therapy use were associated with breast cancer; body mass index <24 with colorectal cancer; alcohol consumption with prostate cancer; and pesticide exposure and body mass index <24 with lung cancer. Physical activity was associated with risk reduction for lung cancer.

Cancer development is a complex and multi-step process, involving multiple risk factors and including environment-gene interactions as determinants of its origin and progression. Although many studies analyzing cancer risk factors have been conducted, reported results vary widely. This may be related to disparities in study designs, geographical features, genetic background, and lifestyle and healthcare factors of the specific populations.[15] In this context, it is important to investigate and clarify risk factors for the most commonly diagnosed cancers regionally, especially manageable factors, so that the best prevention strategies can be formulated.

This study is the first large, matched case-control study of risk factors for common cancers conducted in Brazil. The study site, the UNACON-Bento Goncalves (Cancer Institute of Tacchini Hospital) is a regional health center, where 66% of patients come from the Public Health

Care System located in the far South of Brazil, known as "*Serra Gaúcha*", an important metal-mechanical and winemaking region in the country. Cases include patients diagnosed with the most common cancers diagnosed in this center. Controls were recruited from a variety of community settings in an attempt to represent the population without cancer in this region. Therefore, the data from this study are important and present a picture of the main risk factors already consolidated in the literature for the most diagnosed cancers in the region, which are the same, for the most part, as those diagnosed nationally.

Of nine previous case-control studies undertaken in the Brazilian population, three included breast, colorectal, and lung cancer cases. One study analyzing selected factors associated with breast cancer included 300 women (cases and controls) aged 25-75 years, treated in a single center in Belo Horizonte, Brazil, from 1978 to 1987, and found the following factors to be independently associated with increased risk of breast cancer: parity of less than six deliveries or nulliparity (OR = 5.06, 95% CI: 3.01-8.52 and OR = 2.42, CI: 1.64-3.59, respectively); a history of breast cancer among first degree female relatives (OR = 9.35, 95% CI: 3.22-27.14); and oral contraceptive use (OR = 1.81, 95% CI: 1.15-2.85), which is different from the findings reported here. In another case-control study, including patients with sporadic colorectal adenocarcinomas from Campinas (São Paulo/Brazil) authors did not find a difference in tobacco and alcohol consumption between 169 cases and 101 controls.[7] A third Brazilian case-control study, including 123 lung cancer cases and 123 controls matched by age, sex, and race, done in two medical centers in Rio de Janeiro between 1991 and 1992, found that current and former smoking were associated with OR of 22 (CI: 6.5-7.6) and 7.7 (CI: 2.2-27) for developing lung cancer, respectively. There was no association between cancer risk and occupational exposures.[4]

Premenopausal status was associated with a higher risk of breast cancer in our study. Previous studies have found that postmenopausal women have a lower risk of breast cancer than premenopausal women of the same age and childbearing pattern. Risk increases by almost 3% for each year after menopause onset (natural or surgery induced), and therefore, women who attain menopause at 55 years rather than 45 years, have an approximately 30% higher risk.[16]

Hormone replacement therapy (HRT) was associated with a higher risk of breast cancer in our sample, confirming worldwide evidence from several studies that current and recent users of HRT were at increased risk for breast cancer. Also, a recent study reinforces the importance of HRT as risk factor for breast cancer and concluded that users of systemic hormone therapy who started around the time of menopause were at greater risk of invasive breast cancer than apparently similar never users. Excess risk was greater among current than past users, but some risk persisted for more than a decade after use ceased. There was little excess risk after use of MHT for less than one year, but there were definite excess risks associated with use for one to four years, and progressively greater risks with longer use.[17]

BMI< 24 was associated with higher risk for colorectal and lung cancers in our study. A large previous study evaluated BMI and risk for 22 specific cancers in adults from the UK. For lung, oral cavity, and gastric cancers, low BMI was associated with increased risk, but this risk was driven by current smokers and ex-smokers and was attenuated or disappeared in never smokers.[18] For colorectal cancer, no publication was identified that associated a BMI<24 with an increased risk of developing cancer.

Alcohol consumption seems to have a strong relationship with the development of cancers of the oral cavity, pharynx, esophagus, stomach, colorectum, central nervous system, pancreas, breast, and prostate.[19,20] A cohort study examined the association between alcohol use and prostate cancer among 34,565 men, diagnosed between 50-76 years, and showed that men who consumed more than one drink per month had a small increase in the risk of prostate cancer (hazard ratio, HR = 1.20; 95% CI = 1.02-1.40) compared with men who drank no alcohol or less than one drink per month. Associations between alcohol consumption and prostate cancer are modest and complex. Another study, evaluating the association between alcohol consumption and lung cancer, described a slightly increased risk of lung cancer associated with the consumption of ≥30 g alcohol per day than with no alcohol consumption. Alcohol consumption was strongly associated with increased risk for lung cancer in male never smokers.[21]

Organochlorine and organophosphorus pesticides have been investigated in oxidative stress induction as well as their potential role in cancer development and progression.[21] For lung cancer, occupational pesticide use was associated with the disease in some, but not all, epidemiologic studies. The Agricultural Health Study (AHS) reported positive associations between several pesticides and lung cancer incidence.[23]

The present study confirms the importance of several risk factors for breast, colorectal, prostate, and lung cancers previously associated with these diseases as: tobacco consumption, a first or second-degree family history of cancer, and a family history of cancer in patients under 50 years of age were associated with risk for all these cancers.

In several metanalyses, smoking was significantly associated with lung, prostate, and colorectal cancer incidence and mortality.[24,25,26] A meta-analysis of case-control and cohort studies on tobacco smoking and breast cancer occurrence confirmed consistent evidence for a moderate increase in the risk of breast cancer in women who smoke tobacco.[27]

Finally, about the finding, in our study, associating cancer cases and a first or second-degree family history of cancer and a family history of cancer in patients under 50 years of age, it is known that positive family histories for cancer in general are associated with increased risk for developing the disease and are recognized indicators of high-risk individuals. Individuals reporting an affected relative with certain cancers are at increased risk of developing cancers themselves. The actual risks associated with a positive family cancer history are highly dependent on both the number of affected relatives, degree of relationship and age at which an affected relative was diagnosed. Our study reinforces the importance of evaluation of this risk factor for clinical management, which needs to be considered, always, as a part of the medical evaluation of oncologic patients.).

Additionally, the burden of heredity in the region proved to be relevant. Approximately one in three women with breast cancer and one in five women or men with colorectal cancer, diagnosed under 50 years of age, had a pathogenic or likely pathogenic germline variant. Furthermore, a significant percentage of the individuals analyzed had a VUS, and current studies which will need to be reassessed periodically to verify their role in the disease. All breast or colorectal cancers referred to MGPT met at least one criterion for hereditary cancer predisposition syndromes, reinforcing the need for a detailed assessment of the family history in this region for those diagnosed with cancer, especially when the diagnosis occurs at a young age.

By 2040, the International Agency for Research on Cancer projected the number of cancer cases in South America to increase by 76.5%, and cancer-related deaths by 91.2%.[28] Among the cancer risk factors analyzed here, there are several manageable factors that may contribute to preventive strategies, crucial to optimize cancer control, and for prevention opportunities in this and in other similar communities, in which there are difficulties or lack of access to cancer care. Primary prevention and cancer screening programs, especially for breast and colorectal cancers, are the most cost-effective means to reduce the burden of cancer in Latin America.[29] Future randomized large-scale prospective studies are needed to confirm these issues and to develop a more robust screening model to identify individuals at high-risk for developing cancer and to predict more effectively those which will be affected. A personalized approach, based on individual risk factors, including environmental, behavioral, and genetic risk factors, may help to implement more equitable access to cancer prevention, especially in underserved populations.

**CONTRIBUTORS**

JG and RP conceptualized this study, analyzed the data, wrote, edited, and reviewed all sections, and approved the final version for submission. FMO conceptualized this study, edited, and reviewed all sections, and approved the final version for submission. SC, CMB, MIP and TC were involved in the acquisition of data, reviewed all sections, and approved the final version for submission. CR, GMS and HB were involved in the molecular analysis, reviewed all sections, and approved the final version for submission. PAP was involved in the design of the genetic analysis, wrote, edited, and reviewed all sections, and approved the final version for submission. JRG was involved in the design of the work, wrote, edited, and reviewed all sections, and approved the final version for submission.

**DECLARATIONS OF INTEREST**

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The other authors declare no competing interests.

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**DATA SHARING STATEMENT**

The data sharing from this study will be made available to associate researchers for subprojects that are approved by the Research Ethics Committee of Hospital Tacchini. The study protocol and statistical analysis plan are available upon request to the investigators of this study.

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**Table 1. Socio-demographic characteristics among cases and matched controls.**

| | Cases n (%) | | Controls n (%) | |
|---|---|---|---|---|
| | Female | Male | Female | Male |
| Age Group (years) | | | | |
| 10-19 | 0 (0.0) | 1 (0.2) | 0 (0.0) | 1 (0.2) |
| 20-29 | 12 (2.2) | 14 (3.1) | 12 (2.2) | 14 (3.1) |
| 30-39 | 34 (6.1) | 16 (3.5) | 34 (6.1) | 16 (3.5) |
| 40-49 | 99 (17.8) | 36 (7.9) | 99 (17.8) | 36 (7.9) |
| 50-59 | 123 (22.2) | 100 (22.1) | 123 (22.2) | 100 (22.1) |
| 60-69 | 173 (31.2) | 167 (36.9) | 173 (31.2) | 167 (36.9) |
| 70-79 | 83 (15.0) | 98 (21.6) | 83 (15.0) | 98 (21.6) |
| 80-89 | 30 (5.4) | 21 (4.6) | 30 (5.4) | 21 (4.6) |
| Educational Level (years) | | | | |
| <8 | 235 (43.4) | 240 (54.3) | 45 (8.4) | 37 (8.5) |
| 8-10 | 86 (15.9) | 66 (14.9) | 189 (35.3) | 163 (37.3) |
| 11 | 135 (24.9) | 96 (21.7) | 176 (32.3) | 149 (34.1) |
| >11 | 85 (15.7) | 40 (9.0) | 125 (23.4) | 88 (20.1) |
| Ethnicity (Self Reported) | | | | |
| White | 466 (84.1) | 393 (86.8) | 532 (96.0) | 443 (97.8) |
| Others | 3 (0.5) | 4 (0.9) | 0 (0.0) | 1 (0.2) |
| Marital Status | | | | |
| Single | 76 (13.7) | 73 (16.1) | 48 (8.7) | 29 (6.4) |
| Married | 311 (56.1) | 308 (68.0) | 413 (74.5) | 378 (83.4) |
| Divorced/Widow | 20 (3.6) | 17 (3.8) | 14 (2.5) | 8 (1.8) |

**Table 2. Age at diagnosis among cases by Cancer type.**

| | All Types | | | Colorectal | | | Lung | | | Prostate | Breast | | | Others Types | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Males | Females | Total | Males | Females | Total | Males | Females | Males | Total | Males | Females | Total | Males | Females |
| Cases | 1007 (100.0) | 453 (45.0) | 554 (55.0) | 147 (14.6) | 77 (52.4) | 70 (47.6) | 89 (8.8) | 60 (67.4) | 29 (32.6) | 132 (13.1) | 311 (30.9) | 4 (1.3) | 307 (98.7) | 328 (32.6) | 180 (54.9) | 148 (45.1) |
| Mean (SD) | 59.9 (13.2) | 60.6 (0.6) | 62.6 (0.6) | 57.7 (1.2) | 65.2 (1.1) | 56.7 (1.8) | 65.5 (1.0) | 66.9 (1.2) | 62.5 (2.0) | 68.4 (0.7) | 52.8 (0.8) | 51.5 (10.0) | 52.8 (0.8) | 59.4 (0.7) | 54.7 (0.9) | 65.2 (1.1) |
| Median (Q1-Q3) | 61.0 (51-59) | 62 (54-69) | 62 (54-69) | 60 (11-68.5) | 66 (60-74) | 60 (47-68) | 67 (58-72) | 69 (60.2-73) | 62 (57-69) | 70(62-74) | 52 (42-63) | 51 (31-67) | 52 (42-62.5) | 62 (52-67) | 58 (50-63) | 66 (60-74) |
| Min-Max Age-Groups | 19-89 | 11-89 | 20-87 | 11-89 | 20-86 | 11-89 | 40-87 | 47-87 | 40-82 | 40-88 | 24-87 | 31-72 | 24-87 | 19-86 | 19-71 | 20-86 |
| 10-19 | 1 (0.0) | 1 (0.2) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.3) | 1 (0.5) | 0 (0.0) |
| 20-29 | 26 (2.6) | 14 (3.1) | 12 (2.2) | 4 (2.7) | 3 (3.9) | 1 (1.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (1.3) | 0 (0.0) | 4 (1.3) | 18 (5.5) | 11 (6.11) | 7 (4.7) |
| 30-39 | 50 (5.0) | 16 (3.5) | 34 (6.1) | 6 (4.1) | 5 (6.5) | 1 (1.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 33 (10.6) | 0 (0.0) | 33 (10.7) | 11 (3.3) | 11 (6.11) | 0 (0.0) |
| 40-49 | 135 (13.4) | 36 (7.9) | 99 (17.9) | 22 (15.0) | 12 (15.6) | 10 (14.3) | 7 (7.9) | 3 (5.0 | 4 (13.8) | 1 (7.6) | 84 (27.0 | 2 (50.0) | 82 (26.7) | 21 (6.4) | 18 (10.0) | 3 (2.0) |
| 50-59 | 223 (22.1) | 100 (22.1) | 123 (22.2) | 38 (25.8) | 18 (23.4) | 20 (28.6) | 18 (20.2) | 12 (20.0) | 6 (20.7) | 14 (10.6) | 82 (26.3) | 0 (0.0) | 82 (26.7) | 71 (21.6) | 56 (31.1) | 15 (10.1) |
| 60-69 | 340 (33.8) | 167 (36.9) | 173 (31.2) | 48 (32.6) | 24(31.2) | 24 (34.3) | 29 (32.6) | 17 (28.3) | 12 (41.4) | 44 (33.3) | 69 (22.2) | 1 (25.0) | 68 (22.1) | 150 (45.7) | 81 (45.0) | 69 (46.6) |
| 70-79 | 181 (18.0) | 98 (21.6) | 83 (15.0) | 21 (14.3) | 11 (14.3) | 10 (14.3) | 27 (30.4) | 23 (38.3) | 4 (13.8) | 61 (46.2) | 33 (10.6) | 1 (25.0) | 32 (10.4) | 39 (11.9) | 2 (1.11) | 37 (25.0) |
| 80-89 | 51 (5.1) | 21 (4.6) | 30 (5.4) | 8 (5.4) | 4 (5.2) | 4 (5.7) | 8 (9.0) | 5 (8.3) | 3 (10.3) | 12 (9.1) | 6 (1.9) | 0 (0.0) | 6 (1.9) | 17 (5.2) | 0 (0.0) | 17 (11.5) |

**Other cancers: Non-Hodgkin's lymphoma (n=47; 14.3%); esophagus (n=28; 8.5%), larynx (n= 22; 6.7%), stomach (n=21; 6.4%), pancreas (n=17; 5.2%), central nervous system (n=17; 5.2%), melanoma (n=16; 4.9 %), ovary (n=16; 4.9%), skin (n=15; 4.6%), unknown primary site (n=15; 4.6%), bladder (n=14; 4.3%), sarcoma (n=13; 4.0%), endometrium (n=12; (3.7%), hodgkin's lymphoma (n=11; 3.4%), oropharynx (n=9; 2.7%), cervix (n=6; 1.8%), hypopharynx (n=6; 1.8%), myeloma (n=6; 1.8%), pharynx (n=5; 1.5%), testis (n=5; 1.5%), tongue (n=4; 1.2%), acute myeloid leukemia (n=4; 1.2%), chronic lymphocytic leukemia (n=3; 0.9%), tonsil (n=2; 0.6%), oral cavity (n=2; 0.6%), acute lymphocytic leukemia (n=2; 0.6%), kidney (n=2; 0.6%), ovary + endometrium n=1; (0.3%), ovary + breast (n=1; 0.3%) , liver (n=1; 0.3%), nasopharynx (n=1; 0.3%), parotid (n=1; 0.3%), penis (n=1; 0.3%), thyroid (n=1; 0.3%), and vagina (n=1; 0.3%).*

**Table 3. Associations between risk factors and cancer (overall and according to cancer types).**

| | Cases | Controls | Unadjusted | | Adjusted | |
|---|---|---|---|---|---|---|
| | n=1007 | n=1007 | OR (95%CI) | pvalue | OR (95%CI) | pvalue |
| **All Canceres (n=1007)** | | | | | | |
| Family history of cancer in 1st or 2nd degrees | | | | | | |
| Yes | 723 (72.9) | 320 (31.9) | 6.7 (5.4-8.2) | <0.001 | 6.1 (4.7-7.9) | <0.001 |
| No | 269 (27.1) | 683 (68.1) | 1.0 | | 1.0 | |
| Tobacco Consumption | | | | | | |
| Yes | 413 (44.2) | 87 (8.8) | 9.5 (7.2-12.5) | <0.001 | 8.8 (6.2-12.5) | <0.001 |
| No | 520 (55.7) | 903 (91.2) | 1.0 | | 1.0 | |
| Alcohol Consumption | | | | | | |
| Yes | 133 (15.2) | 13 (1.4) | 14.4 (8.0-25.9) | <0.001 | 9.0 (4.3-19.1) | <0.001 |
| No | 743 (84.8) | 935 (98.6) | 1.0 | | 1.0 | |
| Pesticides Exposures | | | | | | |
| Yes | 222 (23,5) | 67 (6,8) | 4.5 (3.3-6.1) | <0.001 | 2.9 (1.9-4.4) | <0.001 |
| No | 724 (76,5) | 925 (93,2) | 1.0 | | 1.0 | |
| Solvents/Glues Exposures | | | | | | |
| Yes | 76 (8.2) | 19 (2.0) | 3.9 (2.3-6.5) | <0.001 | 1.9 (1.0-4.3) | 0.04 |
| No | 855 (91.8) | 921 (97.9) | 1.0 | | 1.0 | |
| Phisical Activity | | | | | | |
| Yes | 256 (27.3) | 311 (31.1) | 0.9 (0.7-1.1) | 0.28 | | |
| No | 683 (72.7) | 690 (68.9) | 1.0 | | | |
| Body Mass Index | | | | | | |
| <24 | 281 (30.8) | 235 (23.5) | 1.4 (1.1-1.7) | 0.01 | 1.5 (1.1-2.1) | 0.009 |
| 24-28 | 302 (33.1) | 344 (34.4) | 1.0 | | 1.0 | |
| >28 | 330 (36.1) | 421 (42.1) | 0.9 (0.7-1.1) | 0.18 | 0.9 (0.6-1.1) | 0.35 |
| **Breast (n=311)** | | | | | | |
| Family history of cancer in 1st or 2nd degrees | | | | | | |
| Yes | 240 (80.0) | 1175(37.4) | 7.6 (5.1-11.1) | <0.001 | 6.2 (4.1-9.5) | <0.001 |
| No | 60 (19.7) | 192 (62.5) | 1.0 | | 1.0 | |
| Tobacco Consumption | | | | | | |
| Yes | 77 (28.8) | 28 (9.2) | 4.2 (2.6-6.8) | <0.001 | 4.2 (2.4-7.5) | <0.001 |
| No | 190 (71.1) | 277 (90.8) | 1.0 | | 1.0 | |
| Alcohol Consumption | | | | | | |
| Yes | 6 (2.3) | 0 (0.0) | | | | |
| No | 250 (97.7) | 286 (100.0) | | | | |
| Pesticides Exposures | | | | | | |
| Yes | 31 (11.3) | 18 (6.0) | 1.8 (1.0-3.3) | 0.06 | | |
| No | 242 (88.4) | 282 (94.0) | 1.0 | | | |
| Solvents/Glues Exposures | | | | | | |
| Yes | 13 (4.8) | 5 (1.8) | 2.5 (0.9-7.3) | 0.08 | | |
| No | 257 (95.2) | 273 (98.2) | 1.0 | | | |
| Physical Activity | | | | | | |
| Yes | 99 (36.8) | 110 (35.8) | 1.1 (0.8-1.6) | 0.44 | | |
| No | 170 (63.2) | 197 (64.2) | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Body mass Index | | | | | | |
| <24 | 55 (20.7) | 90 (29.4) | 0.7(0.4-1.1) | 0.08 | | |
| 24-28 | 99 (37.3) | 106 (34.6) | 1.0 | | | |
| >28 | 111 (41.9) | 110 (35.9) | 1.0 (0.7-1.5) | 0.94 | | |
| Menarche | | | | | | |
| < 12 years old | 47 (17.5) | 37 (12.5) | 1.5 (1.0-2.5) | 0.07 | | |
| ≥ 12 years old | 221 (82.5) | 260 (87.5) | 1.0 | | | |
| Menopause | | | | <0.001 | | |
| pre-menopausal | 138 (45.0) | 109 (35.5) | 2.8 (1.6-4.8) | | | |
| post-menopausal | 169 (55.0) | 198 (64.5) | 1.0 | | | |
| Gestation | | | | | | |
| Yes | 182 (68.7) | 184 (60.1) | 1.4(1.0-2.0) | 0.08 | | |
| No | 83 (31.3) | 122 (39.9) | 1.0 | | | |
| Abortion | | | | | | |
| Yes | 51 (20.0) | 32 (11.0) | 1.9 (1.2-3.0) | 0.01 | | |
| No | 204 (80.0) | 258 (88.9) | 1.0 | | | |
| Contraceptive use | | | | | | |
| Yes | 190 (71.7) | 213 (74.7) | 0.9 (0.6-1.3) | 0.58 | | |
| No | 75 (28.3) | 72 (25.3) | 1.0 | | | |
| Hormone Replacement Therapy | | | | | | |
| Yes | 25 (10.3) | 10 (3.6) | 3.8 (1.7-8.6) | 0.001 | 3.0 (1.2-7.6) | 0.02 |
| No | 218 (89.7) | 272 (96.4) | 1.0 | | | |
| **Colorectal (n=147)** | | | | | | |
| Family history of cancer in 1st or 2nd degrees | | | | | | |
| Yes | 109 (74.7) | 60 (40.8) | 4.9 (2.8-8.4) | <0.001 | 4.7 (2.6-8.6) | <0.001 |
| No | 37 (25.3) | 87 (59.2) | 1.0 | | 1.0 | |
| Tobacco Consumption | | | | | | |
| Yes | 41 (31.8) | 18 (12.4) | 3.3 (1.7-6.3) | <0.001 | 3.1 (1.5-6.3) | 0.002 |
| No | 88 (68.2) | 127 (87.6) | 1.0 | | 1.0 | |
| Alcohol Consumption | | | | | | |
| Yes | 17 (13.8) | 0 (0.0) | | | | |
| No | 106 (86.2) | 134 (100.0) | | | | |
| Pesticides Exposures | | | | | | |
| Yes | 25 (19.1) | 18 (12.3) | 1.9 (0.9-3.9) | 0.08 | | |
| No | 106 (80.9) | 128 (87.7) | 1.0 | | | |
| Solvents/Glues Exposures | | | | | | |
| Yes | 11 (8.7) | 6 (4.3) | 1.5 (0.5-4.5) | 0.43 | | |
| No | 115 (91.3) | 133 (95.7) | 1.0 | | | |
| Physical Activity | | | | | | |
| Yes | 38 (29.0) | 57 (39.3) | 0.7 (0.4-1.3) | 0.25 | | |
| No | 93 (71.0) | 88 (60.7) | 1.0 | | | |
| Body mass Index | n=913 | n=1007 | | | | |
| <24 | 47 (37.6) | 31 (21.7) | 2.1 (1.1-4.1) | 0.01 | 2.1 (1.0-4.3) | 0.04 |
| 24-28 | 37 (29.6) | 52 (36.4) | 1.0 | | | |
| >28 | 41 (32.8) | 60 (41.9) | 0.9 (0.5-1.6) | 0.18 | 0.7 (0.3-1.3) | 0.24 |
| **Prostate (n=132)** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Family history of cancer in 1st or 2nd degrees | | | | | | |
| Yes | 78 (59.1) | 22 (16.7) | 9.1 (4.8-17.4) | <0.001 | 6.7 (2.8-15.5) | <0.001 |
| No | 54 (40.9) | 110 (83.3) | 1.0 | | 1.0 | |
| Tobacco Consumption | | | | | | |
| Yes | 75 (57.7) | 9 (7.1) | 22.4 (9.7-51.8) | <0.001 | 10.5 (4.2-26.3) | <0.001 |
| No | 55 (42.3) | 118 (92.9) | 1.0 | | 1.0 | |
| Alcohol Consumption | | | | | | |
| Yes | 30 (26.8) | 2 (1.5) | 23.4 (5.4-100.7) | <0.001 | 7.3 (1.3-40.5) | 0.01 |
| No | 82 (73.2) | 128 (98.5) | 1.0 | | 1.0 | |
| Pesticides Exposures | | | | | | |
| Yes | 49 (37.4) | 1 (0.8) | | | | |
| No | 82 (62.6) | 130 (99.2) | | | | |
| Solvents/Glues Exposures | | | | | | |
| Yes | 12 (9.2) | 1 (0.8) | 11.4 (1.4-89.9) | 0.02 | | |
| No | 118 (90.8) | 130(99.2) | 1.0 | | | |
| Physical Activity | | | | | | |
| Yes | 28 (21.5) | 24 (18.3) | 1.2 (0.6-2.3) | 0.52 | | |
| No | 102 (78.5) | 107 (81.7) | 1.0 | | | |
| BMI | | | | | | |
| <24 | | | 1.1 (0.6-2.4) | 0.71 | | |
| 24-28 | | | 1.0 | | | |
| >28 | | | 1.4 (0.8-2.4) | 0.42 | | |
| **Lung (n=89)** | | | | | | |
| Family history of cancer in 1st or 2nd degrees | | | | | | |
| Yes | 60 (67.4) | 20 (22.7) | 8.3 (4.0-17.0) | <0.001 | 30.2 (4.2-218.0) | <0.001 |
| No | 29 (32.6) | 68 (77.3) | 1.0 | | 1.0 | |
| Tobacco Consumption | | | | | | |
| Yes | 75 (85.2) | 8 (9.2) | 115.2 (30.7-432.4) | <0.001 | 1331.9 (48.1-36884.9) | <0.001 |
| No | 13 (14.8) | 79 (90.8) | 1.0 | | 1.0 | |
| Alcohol Consumption | | | | | | |
| Yes | 19 (22.6) | 3 (3.5) | 9.3 (2.6-33.5) | <0.001 | | |
| No | 675 (77.4) | 84 (96.5) | 1.0 | | | |
| Pesticides Exposures | | | | | | |
| Yes | 29 (32.6) | 4 (4.5) | 10.8 (3.5-33.2) | <0.001 | 46.5 (4.1-528.2) | 0.002 |
| No | 60 (67.4) | 85 (95.5) | | | | |
| Solvents/Glues Exposures | | | | | | |
| Yes | 14 (16.1) | 1 (1.1) | 18.1 (2.3-143.6) | <0.001 | | |
| No | 73 (83.9) | 87 (98.9) | | | | |
| Physical Activity | | | | | | |
| Yes | 12 (13.5) | 21 (23.6) | 0.5 (0.2-1.1) | 0.09 | 0.07 (0.01-0.54) | 0.009 |
| No | 77 (86.5) | 68 (76.4) | 1.0 | | | |
| Body mass Index | n=913 | n=1007 | | | | |
| <24 | 281 (30.8) | 235 (23.5) | 2.9 (1.3-6.2) | 0.007 | 9.3 (1.3-67.8) | 0.02 |
| 24-28 | 302 (33.1) | 344 (34.4) | 1.0 | | 1.0 | |
| >28 | 330 (36.1) | 421 (42.1) | 0.6 (0.3-1.2) | 0.17 | 0.9 (0.1-5.0) | 0.86 |

**Table 4. Clinical characteristics and molecular findings in a subgroup of breast and colorectal cancer patients diagnosed under the age of 50 years.**

| Characteristics | Age group | Women | | Men | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| **Breast cancer cases** | | **74** | 97% | **2** | 3% |
| Clinical criteria | | | | | |
| HBOC | | 51 | 69% | 1 | 50% |
| HBOC and Li Fraumeni | | 21 | 28% | - | - |
| HBOC and Lynch | | 1 | 1% | - | - |
| Li Fraumeni | | 1 | 1% | - | - |
| None | | | | 1 | 50% |
| Age at diagnosis (years) | | | | | |
| Mean | 26 - 48 | | | | |
| Median | 39 | | | | |
| Age group | 20 - 29 | 2 | 3% | - | - |
| | 30 - 39 | 36 | 49% | 2 | 100% |
| | 40 - 49 | 36 | 49% | - | - |
| Genetic testing results | | | | | |
| Presence of pathogenic (PP) / Likely pathogenic (LP) variants | | 24 | 32% | - | - |
| Variant of uncertain significance (VUS) | | 20 | 27% | 1 | 50% |
| None PP/LP or VUS | | 30 | 41% | 1 | 50% |
| **Colorectal cancer cases** | | **17** | 50% | **17** | 50% |
| Clinical criteria | | | | | |
| APC | | - | - | 1 | 6% |
| Li Fraumeni | | - | - | 1 | 6% |
| Lynch | | 15 | 88% | 15 | 88% |
| HBOC | | 1 | 6% | - | - |
| None | | 1 | 6% | - | - |
| Age at diagnosis (years) | | | | | |
| Mean | 36 - 49 | | | | |
| Median | 46 | | | | |
| Age group | 10 - 19 | - | - | 1 | 6% |
| | 20 - 29 | - | - | 2 | 12% |
| | 30 - 39 | 3 | 18% | 5 | 29% |
| | 40 - 49 | 14 | 82% | 9 | 53% |
| Genetic testing results | | | | | |
| Presence of pathogenic (PP) / Likely pathogenic (LP) variants | | 3 | 18% | 4 | 24% |
| Variant of uncertain significance (VUS) | | 4 | 24% | 3 | 18% |
| None PP/LP or VUS | | 10 | 59% | 10 | 59% |

**Table 5. Description of the molecular variants identified**

| Sex | Cancer diagnosed | Age at diagnosis | Criteria | Molecular findings | Classification of the variant |
|---|---|---|---|---|---|
| Woman | Breast | 29 | HBOC, Li Fraumeni | *CHEK2* c.636del (p.Phe212LeufsTer2); *BRIP1* c.388G>A p.(Glu130Lys); *MUTYH* c.167G>T p.(Gly56Val) | Pathogenic variant; VUS; VUS |
| Woman | Breast | 32 | HBOC, Li Fraumeni | *TP53* c.733G>A p.(Gly245Ser); *BRCA1* c.1601A>G p.(Gln534Arg); *PALB2* NC_000016.9:g.23614609_23619445del | Pathogenic variant; VUS; VUS |
| Woman | Breast | 32 | HBOC, Li Fraumeni | *BARD1* c.841C>T p.(Pro281Ser) | VUS |
| Woman | Breast | 32 | HBOC | *BRCA1* c.4675+1G>A; *MRE11A* c.482A>G p.(Lys161Arg) | Pathogenic variant; VUS |
| Woman | Breast | 33 | HBOC, Li Fraumeni | *MUTYH* c.1147delC p.(Ala385ProfsTer23) em heterozygosity | Pathogenic variant |
| Woman | Breast | 33 | HBOC, Li Fraumeni | *CHEK2* c.813-7C>T em heterozygosity | VUS |
| Woman | Breast | 33 | HBOC, Li Fraumeni | *BRCA2* c.5682C>G p.(Tyr1894Ter); *ATM* c.4709T>C p.(Val1570Ala) | Pathogenic variant; VUS |
| Woman | Breast | 33 | HBOC, Li Fraumeni | *BRIP1* c.1586G>A em heterozygosity p.(Gly529Glu); *MSH6* c.334A>G em heterozygosity p.(Asn112Asp); *RAD51D* c.323+2T>C em heterozygosity | VUS; VUS; VUS |
| Woman | Breast | 35 | HBOC | *BRCA2* c.7823C>A (p.Pro2608Gln) | VUS |
| Woman | Breast | 35 | HBOC | *BRCA2* c.8488-1G>A | Likely pathogenic variant |
| Woman | Breast | 35 | HBOC, Li Fraumeni | *ATM* c.8428A>C (p.Lys2810Gln) em heterozygosity | VUS |
| Woman | Breast | 35 | HBOC, Li Fraumeni | *ATM* c.790del em heterozygosity p.(Tyr264IlefsTer12) | Pathogenic variant |
| Woman | Breast | 35 | HBOC, Li Fraumeni | *CHEK2* c.497A>G em heterozygosity p.(Asn166Ser) | VUS |
| Woman | Breast | 36 | HBOC | *BRCA2* c.7180A>T p.(Arg2394Ter) | Pathogenic variant |
| Woman | Breast | 36 | HBOC | *BARD1* c.1758del em heterozygosity p. (Ser586ArgfsTer5); *MSH2* c.376G>A em heterozygosity p.(Gly126Ser); *CHEK2* c.1711G>A em heterozygosity p.(Glu571Lys) | Pathogenic variant; VUS; VUS |
| Woman | Breast | 36 | HBOC | *BRCA2* c.9101A>G p.(Gln3034Arg); *MSH2* c.2726G>A p.(Arg909Gln) | VUS; VUS |

| | | | | | |
|---|---|---|---|---|---|
| Woman | Breast | 37 | HBOC | *ATM* c.8021C>T em heterozygosity p.(Thr2674Ile) | VUS |
| Woman | Breast | 37 | HBOC | *CHEK2* c.599T>C p.(Ile200Thr); *MUTYH* c.1187G>A p.(gly396Asp) | Pathogenic variant; VUS |
| Woman | Breast | 37 | HBOC | *BRCA1* c.798_799del em heterozygosity p.(Ser267LysfsTer19) | Pathogenic variant |
| Woman | Breast | 38 | HBOC, Li Fraumeni | *BRCA1* c.379delA p.(Ser127ValfsTer36); *BRCA1* c.521A>C p.(Gln174Pro) | Pathogenic variant; VUS |
| Woman | Breast | 39 | HBOC | *RAD51D* c.394G>A em heterozygosity p.(Val132Ile) | VUS |
| Woman | Breast | 39 | HBOC | *ATM* c.8814_8824del p.(Met2938IlefsTer14) | Pathogenic variant |
| Woman | Breast | 39 | HBOC | *ATM* c.7408T>G p.(Tyr2470Asp); *CHEK2* c.1423T>A p.(Phe475Ile) | Pathogenic variant; VUS |
| Woman | Breast | 40 | HBOC | *MSH6* c.1829A>G p.(Lys610Arg) | VUS |
| Woman | Breast | 40 | HBOC | *ATM* c.1273G>T p.(Ala425Ser) | VUS |
| Woman | Breast | 40 | HBOC | *MUTYH* c.505-2A>C em heterozygosity | Likely pathogenic variant |
| Woman | Breast | 41 | HBOC | *BRCA2* c.9367A>G em heterozygosity p.(Ser3123Gly) | VUS |
| Woman | Breast | 41 | HBOC | *BRCA1* c.4183C>T em heterozygosity p.(Gln1395Ter) | Pathogenic variant |
| Woman | Breast | 41 | HBOC | *BLM* c.2695C>T p.(Arg899Ter) em heterozygosity | Pathogenic variant |
| Woman | Breast | 42 | HBOC | *BRCA2* c.5687C>T em heterozygosity p.(Ala1896Val) | VUS |
| Woman | Breast | 42 | HBOC | *ATM* c.6572+4T>C; *RAD51C* c.431T>C p.(Ile144Thr) | VUS; VUS |
| Woman | Breast | 42 | HBOC, Li Fraumeni | *BRCA2* c.5682C>G p.(Tyr1894Ter); *MLH1* c.654_655invCA p.(Ile219Val) | Pathogenic variant; VUS |
| Woman | Breast | 42 | HBOC | *RAD50* c.2467C>G p.(Arg823Gly) | VUS |
| Woman | Breast | 42 | HBOC | *BRIP1* c.2392C>T em heterozygosity p.(Arg798Ter); *BRCA1* c.5509T>C em heterozygosity p.(Trp1837Arg) | Pathogenic variant; likely pathogenic variant |
| Woman | Breast | 43 | HBOC | *MSH6* c.34C>A em heterozygosity p.(Pro12Thr) | VUS |
| Woman | Breast | 43 | HBOC | *MSH6* c.3438+6T>C em heterozygosity; *CDH1* c.118A>G em heterozygosity p.(Thr40Ala) | VUS; VUS |

| | | | | | |
|---|---|---|---|---|---|
| Woman | Breast | 44 | HBOC | *BARD1* (c.2255A>G) p.(Gln752Arg); *BLM* (c.956T>G) p.(leu319Arg) | VUS; VUS |
| Woman | Breast | 44 | HBOC | *TP53* c.1010G>A em heterozygosity p.(Arg337His); *BRCA2* c.811G>A em heterozygosity p.(Gly271Arg) | Pathogenic variant; VUS |
| Woman | Breast | 45 | HBOC | *TP53* c.1010G>A p.(Arg337His) | Pathogenic variant |
| Woman | Breast | 45 | HBOC, Lynch | *ATM* c.6820G>A p.(Ala2274Thr); *ATM* c.6871T>C p.(Trp2291Arg) | VUS; VUS |
| Woman | Breast | 46 | HBOC | *BRCA1* c.1612C>T p.(Gln538Ter) | Pathogenic variant |
| Woman | Breast | 47 | HBOC, Li Fraumeni | *MUTYH* c.1147delC p.(Ala385ProfsTer23); *RAD51D* c.728-7_728-5del | Pathogenic variant; VUS |
| Woman | Breast | 48 | HBOC | *BRCA1* c.1612C>T p.(Gln538Ter) | Pathogenic variant |
| Woman | Breast | 48 | Li Fraumeni | *ATM* c.3800A>T em heterozygosity p.(Glu1267Val); *BRCA2* c.9203C>T em heterozygosity p.(Ser3068Phe) | VUS; VUS |
| Man | Breast | 31 | HBOC | *ATM* c.5999G>T p.(Ser2000Ile) | VUS |
| Woman | Colorectal | 47 | Lynch | *XRCC2* c.574T>C p.(Phe192Leu) | VUS |
| Woman | Colorectal | 40 | Lynch | *MUTYH* c.949C>T p.(Leu317Phe) | VUS |
| Woman | Colorectal | 48 | Lynch | *MRE11A* c.502A>T p.(Ser168Cys) | VUS |
| Woman | Colorectal | 36 | Lynch | *EPCAM-MSH2del*; *PMS2* c.1243G>A p.(Val415Met) | Pathogenic variant; VUS |
| Woman | Colorectal | 47 | Lynch | *CHEK2* c.599T>C em heterozygosity p.(Ile200Thr); *BRIP1* c.550G>T em heterozygosity p.(Asp184Tyr) | Pathogenic variant; VUS |
| Woman | Colorectal | 36 | Lynch | *MEN1* c.1655A>G em heterozygosity p.(Glu552Gly) | VUS |
| Woman | Colorectal | 48 | Lynch | *MUTYH* c.481G>C p.(Asp161His); *TP53* NC_000017.10:g.7579264_7579750del | VUS; pathogenic variant (not confirmed by MLPA) |
| Man | Colorectal | 30 | APC | *MSH6* c.1730G>A p.(Arg577His) | VUS |
| Man | Colorectal | 45 | Lynch | *BRCA1* c.379delA p.(Ser127ValfsTer36); *BRCA1* c.521A>C p.(Gln174Pro) | Pathogenic variant; VUS |
| Man | Colorectal | 49 | Lynch | *MUTYH* c.452A>G em heterozygosity p.(Tyr151Cys) | Pathogenic variant |
| Man | Colorectal | 48 | Lynch | *MUTYH* c.193C>T p.(Pro65Ser) | VUS |

| | | | | | |
|---|---|---|---|---|---|
| Man | Colorectal | 33 | Lynch | *MSH6* c.3991C>T p.(Arg1331Ter)BRIP1 c.344C>A p.(Asp1148Glu); | Pathogenic variant; VUS |
| Man | Colorectal | 47 | Lynch | *XRCC2* c.283A>G p.(Ile95Val) | VUS |
| Man | Colorectal | 44 | Lynch | *MLH1* NC_000003.11:g.37089870_37092271del; *MSH6* c.3758T>A p.(Val1253Glu) | Likely pathogenic variant; VUS |

**Supplementary table 1. List of Genes analyzed by the germline multigene cancer panel and their association with breast and colorectal cancer predisposition**

| Gene | NM code | Breast cancer | Colorectal cancer |
|---|---|---|---|
| *BRCA1* | NM_007294.2 | *** | Controversial |
| *BRCA2* | NM_000059.3 | *** | Controversial |
| *CDH1* | NM_004360.4 | *** | Unrelated |
| *EPCAM* | NM_002354.2 | * | * |
| *MEN1* | NM_000244.3 | Unrelated | Unrelated |
| *MLH1* | NM_000249.3 | Controversial | *** |
| *MSH2* | NM_000251.2 | Controversial | *** |
| *MSH6* | NM_000179.2 | Controversial | *** |
| *MUTYH* | NM_001128425.1 | Controversial | *** |
| *FAM175A* | | | |
| *PALB2* | NM_024675.3 | *** | Unrelated |
| *PMS2* | NM_000535.5 | Controversial | Controversial |
| *PTEN* | MN_000314.4 | *** | * |
| *STK11* | NM_000455.4 | *** | ** |
| *TP53* | NM_000546.5 | *** | ** |
| *ATM* | NM_000051.3 | ** | Controversial |
| *BRIP1* | NM_032043.2 | * | Unrelated |
| *CHEK2* | NM_007194.3 | ** | * |
| *NBN* | NM_002485.4 | ** | Unrelated |
| *RAD51C* | NM_058216.2 | * | Unrelated |
| *RAD51D* | NM_002878.3 | * | Unrelated |
| *BARD1* | NM_000465.2 | * | Unrelated |
| *BLM* | NM_000057.2 | * | * |
| *MRE11* | NM_00591.3 | * | Unrelated |
| *RAD50* | NM_005732.3 | * | Unrelated |
| *XRCC2* | NM_005431.1 | * | Unrelated |

High Risk (***)

Moderate Risk (**)

Low Risk or Insufficient data (*)

**Supplementary table 2. Pesticides used among cases and controls described by chemical types.**

| Chemical types | Case n (%) | Control n (%) |
|---|---|---|
| **Fungicides** | **25 (11.6)** | **8 (16.0)** |
| One type | 12 | 7 |
| 2 types | 8 | 1 |
| 3 or more types | 5 | 0 |
| **Herbicides** | **58 (27.0)** | **16 (32.0)** |
| One type | 32 | 12 |
| 2 types | 15 | 3 |
| 3 or more types | 11 | 1 |
| **Insecticides-agriculture** | **4 (1.9)** | **1 (2.0)** |
| One type | 2 | 1 |
| 2 types | 1 | 0 |
| 3 or more types | 1 | 0 |
| **Fungicides and insecticides** | **4 (1.9)** | **1 (2.0)** |
| **Fungicides and herbicides** | **40 (18.6)** | **9 (18.0)** |
| **Insecticides and herbicides** | **2 (0.9)** | **1 (2.0)** |
| **Fungicides, insecticides and herbicides** | **7 (3.3)** | **1 (2.0)** |
| **Trade name/class of product not cited, and report use of various types** | **75 (34.9)** | **13 (26.0)** |
| | 215 | 50 |