Association of PIN3 16-bp Duplication Polymorphism of *TP53* gene with Breast Cancer Risk in Mali and A Meta-analysis.

Brehima Diakite (✉ br.diakite@yahoo.fr)  
University of Sciences, Techniques and Technologies of Bamako  
https://orcid.org/0000-0001-8296-5292

Yaya Kassogue  
University of Sciences, Techniques and Technologies of Bamako

Guimogo Dolo  
University of Sciences, Techniques and Technologies of Bamako

Oumar Kassogue  
University of Sciences, Techniques and Technologies of Bamako

Mamadou Lassine Keita  
University Teaching Hospital Point G

Brian Joyce  
Cancer Epidemiology and Prevention Northwestern University

Erin Neuschler  
University of Illinois at Chicago

Jun Wang  
Northwestern University

Jonah Musa  
Northwestern University

Cheick Bougari Traore  
University of Sciences, Techniques and Technology Bamako

Bakarou Kamate  
University of Sciences, Techniques and Technologies of Bamako

Etienne Dembele  
Northwestern University

Nadifi Sellama  
Hassan II University

Mercy Isichei  
University of Jos

Jane L Holl  
University of Chicago
Robert Murphy  
Institute For Global Health, Northwestern University  

Seydou Doumbia  
University of Sciences, Techniques and Technologies of Bamako  

Lifang Hou  
Northwestern University  

Mamoudou Maiga  
Northwest University  

Research article

Keywords: Breast cancer, TP53, PIN316-bp duplication, Meta-analysis, Malian population

Posted Date: June 4th, 2020

DOI: https://doi.org/10.21203/rs.2.19205/v3

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License

Version of Record: A version of this preprint was published at BMC Medical Genetics on July 3rd, 2020.  
See the published version at https://doi.org/10.1186/s12881-020-01072-4.
Abstract

Background. Breast cancer, the most common tumor in women in Mali and worldwide has been linked to several risk factors, including genetic factors, such as the PIN3 16-bp duplication polymorphism of TP53. The aim of our study was to evaluate the role of the PIN3 16-bp duplication polymorphism in the susceptibility to breast cancer in the Malian population and to perform a meta-analysis to better understand the correlation with data from other populations.

Methods. We analyzed the PIN3 16-bp duplication polymorphism in blood samples of 60 Malian women with breast cancer and 60 healthy Malian women using PCR. In addition, we performed a meta-analysis of case-control study data from international databases, including Pubmed, Harvard University Library, Genetics Medical Literature Database, Genesis Library and Web of Science. Overall, odds ratio (OR) with 95% CI from fixed and random effects models were determined. Inconsistency was used to assess heterogeneity between studies and publication bias was estimated using the funnel plot.

Results. In the studied Malian patients, a significant association of PIN3 16-bp duplication polymorphism with breast cancer risk was observed in dominant (A1A2+A2A2 vs. A1A1: OR = 2.26, CI 95% = 1.08-4.73; P = 0.02) and additive (A2 vs. A1: OR = 1.87, CI 95% = 1.05-3.33; P = 0.03) models, but not in the recessive model (P = 0.38). In the meta-analysis, nineteen (19) articles were included with a total of 6,018 disease cases and 4,456 controls. Except for the dominant model (P = 0.15), an increased risk of breast cancer was detected with the recessive (OR = 1.46, 95% CI = 1.15-1.85; P = 0.002) and additive (OR = 1.11, 95% CI = 1.02-1.19; P = 0.01) models.

Conclusion. The case-control study showed that PIN3 16-bp duplication polymorphism of TP53 is a significant risk factor for breast cancer in Malian women. These findings are supported by data from the meta-analysis carried out on different ethnic groups around the world.

Background

Breast cancer as a multifactorial disease is the most diagnosed cancer among women worldwide [1]. The incidence of breast cancer in women would be higher in developed countries due to the great heterogeneity in terms of polymorphism frequency, proportion of deletions and insertions, but with the recent improvements and availability of diagnostic infrastructure in LMICs, the detection rate has continued to increase. Over the past decade, the number of women globally affected has increased, but data from LMICs are still limited [2]. With the advent of genomics, dramatic advances have been made in breast cancer research. Recent data revealed that in addition to clinical, lifestyle and environmental risk factors, an individual’s genetic background plays a major role in the development of breast cancer [3]. Several genes have been shown to be associated with an increased risk of breast cancer, such as damaged DNA repair genes (BRCA1 and BRCA2), tumor protein p53 (TP53), Checkpoint kinase 2 (CHEK2), methylenetetrahydrofolate reductase (MTHFR), fibroblast growth factor receptor 2 (FGFR2) and glutathione S-transferase mu 1 (GSTM1) [4]. TP53, a tumor suppressor gene, is involved not only in the
development of breast cancer, but also in the development of other human cancers. Indeed, this gene plays a crucial role in the response to stress. The protein TP53, also called the genome guardian, is a transcription factor that controls the expression of many genes involved in cell cycle regulation, DNA repair, cell death and senescence [5-8]. The great heterogeneity reported in the TP53 in breast cancer may be linked to the geographic origin and ethnic differences of patients [8-10].

The TP53 is located on the chromosome 17p13.1 [12] and consists of twelve exons (https://www.ncbi.nlm.nih.gov/gene/7157). It is highly polymorphic both in exonic and intronic regions with more than 200 polymorphisms (http://www-p53.iarc.fr/). Of these, p.Arg72Pro, p.Pro47Ser and PIN3 16-bp duplication of TP53 are the most studied polymorphisms because of their critical roles in modifying the function and/or expression of TP53 [7, 13]. PIN3 16-bp duplication may affect sequence changes in the coding-region, which leads to an impaired p53 function and expression [14]. This disturbance is involved in the etiopathology of many cancers, including breast cancer [15, 16]. Several studies around the world have found an association between the polymorphisms of this gene and the development of breast cancer [17, 18], while others have found no influence [19-21]. It has been reported in developed countries that individuals harboring the A2A2 genotype or 16-bp duplication in intron 3 of TP53 are at increased risk of breast cancer [22, 23]. However, very few studies have been performed in Africa populations [20], especially in Mali. The literature review showed that the relationship between PIN3 16-bp duplication polymorphism and the risk of breast cancer has not been evaluated in our population. Therefore, we carried out the present work in order to understand firstly the relation between PIN3 16-bp duplication and the risk of breast cancer in the Malian population and secondly to perform a comparative meta-analysis of different studies around the world better estimate the risk of breast cancer.

**Methods**

**Case control study**

**Subject selection and Sample collection**

The study was approved by the ethics committee of the Faculty of Medicine and Odontostomatology (2018/63/CE/FMPOS) at the University of Sciences, Techniques and Technologies of Bamako (USTTB). The study was explained to each participant prior being asked to sign the approved Informed Consent.

Sixty women (mean age 43.72±3.14) with clinically and histologically diagnosed breast cancer and 60 age-matched apparently healthy women (mean age 43.90±2.92) from the general population were recruited at the University Hospital Center (CHU) of Point G in Bamako, Mali, between July 2018 and July 2019. All cases had early stage cancer (stage II). Clinico-pathological parameters including age at diagnostic, localization, use of contraceptive, menopausal status, parity, breastfeeding, family history of breast cancer, history of benign breast disease, obesity, smoking, histological type, tumor size, nodal involvement and metastasis were collected from each patient's medical record. In the control group, the inclusion criteria were all Malian women aged of 18 years or over coming from the general population of whom no chronic disease has ever been diagnosed (such as cancer, diabetes, etc.) and having accepted
informed consent. Healthy subjects with a history of breast cancer, chronic diseases such as diabetes, or other types of cancer were excluded as controls. A total Five milliliter of peripheral blood was collected from each participant in an EDTA tube for the genotyping analysis of PIN3 16-bp duplication polymorphism of TP53.

Genotyping of PIN3 16-bp duplication

Qiagen's GentaPuregene Extraction Kit was used to extract the genomic DNA from white blood cells. DNA quantity and quality were determined by spectrophotometer. Genotyping of PIN3 16-bp duplication polymorphism was performed by allele specific PCR (AS-PCR) using published primers previously described [18, 20, 24, 25]. A final reaction volume of 25 μl containing 12.8 μl buffer, 1.5 μl MgCl2, 1.5 μl dNTPs, 1.0 μl primers, 2.0 μl Taq DNA polymerase, and 2.0 μl genomic DNA was used to amplify the PIN3 16-bp duplication of the TP53. PCR amplification conditions were an initial denaturation step for 5 minutes (min) at 95°C, followed by 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 59°C and 1 min extension at 72°C, and a final extension step at 72°C for 5 min. The PCR products after electrophoresis on a 4.5% agarose gel showed a fragment of 119 bp for the A1 allele (wild type or no duplication) and a fragment of 135 bp for the A2 allele (Insert or 16-bp duplication).

Statistical analysis

SPSS 11.0 was used to analyze the data. Chi-square tests (two-sided) were performed to evaluate the correlation between the PIN3 16-bp duplication and the clinical and histological features. Hardy-Weinberg equilibrium for the PIN3 16-bp duplication genotype distribution of TP53 was tested by Chi2 analysis with exact probability. An odds ratio (OR) test with 95% confidence interval (CI) and P ≤0.05 was used to determine the association between PIN3 16-bp duplication polymorphism of TP53 and the risk of breast cancer, according to the different genetic models (dominant: A1A2 + A2A2 vs. A1A1, recessive: A2A2 vs. A1A2 + A1A1 and additive: A2 vs. A1). The P value <0.05 was considered significant.

Meta-analysis Study

Literature Search

The keywords “TP53”, “Intron 3 Ins16 bp or PIN3 16-bp duplication”; “Polymorphism or mutation or genes” and “breast cancer” were used to perform a literature search of Pubmed, Harvard University Library, Genetics Medical Literature Database, Genesis Library and Web of Science. Only articles published in English were retained. Additional articles were identified by examining the references cited in articles and reviews retained from the search.

Article Inclusion Criteria

The criteria for selecting the articles were as follows: (1) Results reported about a case-control study, study published as an original study evaluating the association between PIN3 16-bp duplication polymorphism of TP53 and the risk of breast cancer; (2) No deviation from Hardy-Weinberg Equilibrium
Data Extraction

The following data were extracted from all eligible studies: first author's name, year of publication, study population, sample size, genotypic and allelic distribution by two independent investigators (add the initials of the two extractors). These data were compared to find a consensus. A third investigator resolved any conflict.

Statistical analysis

Review Manager Software was used to analyze the data. The Chi-squared test with the value of $P < 0.05$ was carried out to evaluate the Hardy-Weinberg equilibrium in the controls. The association of PIN3 16-bp duplication polymorphism with the risk of breast cancer in the dominant, recessive and additive models was measured by ORs with 95% CI. An inconsistency ($I^2$) test was performed to detect heterogeneity [26]. If $I^2 \leq 50\%$ (absence of heterogeneity), the fixed effect model (FEM) was chosen as a pooling method; otherwise, if $I^2 > 50\%$ (presence of heterogeneity), the random effect model (REM) was maintained. The addition and/or deletion of any study that modifies the value of the pooled OR ±1 was done to assess the sensitivity of the meta-analysis. The funnel curve was used to identify the publication bias.

Results

Case control Study

We evaluated the association between PIN3 16-bp duplication polymorphism of $TP53$ and the risk of breast cancer in Malian women. The socio-demographic, clinical, and pathological characteristics of the patients are shown in Table 1. The mean age of cases and controls was 43.72±3.14 and 43.90±2.92 years, respectively. Most of patients had cancer in the left breast. Multiparity was reported in 75.5% of cases, breastfeeding in 88.3%, no family history of breast cancer in 86.6%, no history of benign breast disease in 90.0%, absence of obesity in 68.3% and no history of smoking in 88.3% of the cases. Invasive ductal carcinoma forms were more prevalent than any others histological form of breast cancer (Table 1). Patients with PIN3 16-bp duplication (A2A2) of $TP53$ were more likely to have an invasive ductal carcinoma form, T3 stage tumor size, node involvement (N0 and N1), and M0 metastasis status compared to patients with the A1A1 or A1A2 genotype. We found no correlation between the PIN316-bp duplication polymorphism and the clinical features of participants except histological type ($p = 0.04$).

Table 1 Distribution of the PIN3 16-bp duplication polymorphism of $TP53$ according to the clinicopathological characteristics in Malian breast cancer.
| Clinical parameter                        | PIN3 16-bp duplication |
|------------------------------------------|------------------------|
|                                          | N (%)                  | A1A1     | A1A2     | A2A2     | X²      | P value |
| Mean age at diagnostic                   | 43.72±3.14             |          |          |          | 2.41*   | 0.12    |
| ≤ 40 years of age                        | 29 (48.3)              | 11 (37.9)| 12 (41.4)| 6 (20.7) |          |         |
| > 40 years of age                        | 31 (51.7)              | 16 (51.6)| 13 (41.4)| 2 (6.5)  |          |         |
| Localization                             | 1.98                   |          |          |          | 0.74    |         |
| Right breast                             | 19 (31.7)              | 7 (36.8) | 9 (47.4) | 3 (15.8) |          |         |
| Left breast                              | 37 (61.7)              | 19 (51.4)| 14 (37.8)| 4 (10.8) |          |         |
| Bilateral                                | 4 (6.6)                | 1 (25.0) | 2 (50.0) | 1 (25.0) |          |         |
| Use of contraceptives                    | 0.56*                  |          |          |          | 0.45    |         |
| No                                       | 45 (75.0)              | 18 (40.0)| 25 (55.6)| 2 (4.4)  |          |         |
| Yes                                      | 15 (25.0)              | 9 (60.0) | -        | 6 (40.0) |          |         |
| Menopausal status                        | 3.15                   |          |          |          | 0.53    |         |
| Pre-menopausal                           | 11 (18.3)              | 6 (54.5) | 4 (36.4) | 1 (9.1)  |          |         |
| Post-menopausal                          | 20 (33.3)              | 10 (50.0)| 9 (45.0) | 1 (5.0)  |          |         |
| Fertile women                            | 29 (48.3)              | 11 (37.9)| 12 (41.4)| 6 (20.7) |          |         |
| Parity                                   | 7.33                   |          |          |          | 0.12    |         |
| Nulliparity                              | 6 (10.0)               | -        | 5 (83.3) | 1 (16.7) |          |         |
| Primiparity                              | 9 (15.0)               | 3 (33.3) | 4 (44.4) | 2 (22.2) |          |         |
| Multiparity                              | 45 (75.5)              | 24 (53.3)| 16 (35.6)| 5 (11.1) |          |         |
| Breastfeeding                            | 0.50*                  |          |          |          | 0.48    |         |
| Yes                                      | 53 (88.3)              | 26 (49.1)| 19 (35.8)| 8 (15.1) |          |         |
| No                                       | 7 (11.7)               | 1 (14.3) | 6 (85.7) | -        |          |         |
| Family history of BC                     | 0.64*                  |          |          |          | 0.42    |         |
| Yes                                      | 8 (13.3)               | 4 (50.0) | 4 (50.0) | -        |          |         |
| No                                       | 52 (86.7)              | 23 (44.2)| 21 (40.4)| 8 (15.4) |          |         |
| Personal history of benign breast disease| 1.69*                  |          |          |          | 0.19    |         |
| Yes                                      | 6 (10.0)               | 4 (66.7) | 2 (33.3) | -        |          |         |
| No                                       | 54 (90.0)              | 23 (42.6)| 23 (42.6)| 8 (14.8) |          |         |
| Obesity                                  | 0.43                   |          |          |          | 0.81    |         |
| Yes                                      | 19 (31.7)              | 8 (42.1) | 9 (47.4) | 2 (10.5) |          |         |
| No                                       | 41 (68.3)              | 19 (46.3)| 16 (39.0)| 6 (14.6) |          |         |
| Smoking                                  | 0.20*                  |          |          |          | 0.65    |         |
| Passive smoking                          | 7 (11.7)               | 3 (42.9) | 4 (57.1) | -        |          |         |
| No                                       | 53 (88.3)              | 24 (45.3)| 21 (39.6)| 8 (15.1) |          |         |
| Histological type                        | 4.14*                  |          |          |          | 0.04    |         |
| Invasive ductal carcinoma                | 56 (93.3)              | 23 (41.1)| 25 (44.6)| 8 (14.3) |          |         |
| Others                                   | 4 (6.7)                | 4 (100.0)| -        | -        |          |         |
| Tumor size                               | 5.63                   |          |          |          | 0.46    |         |
| T1                                       | 1 (1.7)                | -        | 1 (100.0)| -        |          |         |
| T2                                       | 10 (16.7)              | 5 (50.0) | 5 (50.0) | -        |          |         |
| T3                                       | 41 (68.3)              | 18 (43.9)| 15 (36.6)| 8 (19.5) |          |         |
| T4                                       | 8 (13.3)               | 4 (50.0) | 4 (50.0) | -        |          |         |
| Nodal involvement                        |                        |          |          |          |         |         |
| N0                                       | 36 (60.0)              | 16 (44.4)| 16 (44.4)| 4 (11.1) | 6.05    | 0.41    |
| N1                                       | 16 (26.7)              | 5 (31.3) | 7 (43.8) | 4 (25.0) |          |         |
| N2                                       | 7 (11.7)               | 5 (71.4) | 2 (28.6) | -        |          |         |
Table 2 shows the distribution of PIN3 16-bp duplication polymorphism of the TP53 in the cases according to the genetic models. The genotypic distribution PIN3 16-bp duplication polymorphism did not deviate from the Hardy-Weinberg equilibrium both in the cases ($X^2 = 0.33, p = 0.57$) and in the controls ($X^2 = 2.76, p = 0.10$). The heterozygous genotype (A1A2) was associated with an increased risk of breast cancer with ($OR = 2.25, 95\% CI = 1.01-5.01$ and $p = 0.04$). When we extended the analysis to the different genetic models, we noted that the dominant model (A1A2+A2A2 vs. A1A1: $OR = 2.26, 95\% CI = 1.08-4.73$, $p = 0.02$) and the additive model (A2 vs A1: $OR = 1.87, 95\% CI = 1.05-3.33$, $p = 0.03$) of PIN3 16-bp duplication polymorphism was significantly associated with the risk of breast cancer (Table 2).

### Table 2. Association of genetic models of PIN3 16-bp duplication polymorphism of TP53 with breast cancer risk.

| Genotype/Allele | Cases N=60 | Controls N=60 | OR (95\% CI) | P  |
|-----------------|------------|---------------|--------------|----|
| A1A1            | 27 (45.0)  | 39 (65.0)     | Reference    |    |
| A1A2            | 25 (41.7)  | 16 (26.7)     | 2.25 (1.01-5.01) | 0.04 |
| A2A2            | 8 (13.3)   | 5 (8.3)       | 2.31 (0.68-7.83) | 0.17 |
| A2A2 + A1A2     | 33 (55.0)  | 21 (35.0)     | 2.26 (1.08-4.73) | 0.02 |
| A1A1+A1A2       | 52 (86.7)  | 55 (91.7)     | Reference    |    |
| A2A2            | 8 (13.3)   | 5 (8.3)       | 1.69 (0.52-5.50) | 0.38 |
| A1              | 79 (65.8)  | 94 (78.3)     | Reference    |    |
| A2              | 41 (34.2)  | 26 (21.7)     | 1.87 (1.05-3.33) | 0.03 |

N= Number; CI: confidence Interval; P: p-value; A2A2 + A1A2 vs. A1A1: Dominant model, A2A2 vs. A1A1+A1A2: Recessive model; A2 vs. A1: Additive model.

### Meta-analysis Study

**Characteristics of Included Studies**

A total of 19 articles reporting case-control studies that investigated PIN3 16-bp duplication polymorphism and breast cancer risk and meeting the inclusion criteria (Table 3) were selected to perform the meta-analysis. Thirty studies that have not addressed PIN3 16-bp duplication of TP53, 6
studies deviating from HWE, as well as 2 studies [27, 28] which influenced the OR and p values pooled were excluded (Figure 1).

Table 3 Summary of studies included in meta-analysis.

| Reference                     | Population | Cases |            |            |            |            | Controls |            |            |            |            |            | HWE  |
|-------------------------------|------------|-------|------------|------------|------------|------------|----------|------------|------------|------------|------------|------------|------|
| Present study                 | Mali       | N     | A1A1       | A1A2       | A2A2       | N           | A1A1     | A1A2       | A2A2       |            |            |            | 0.10 |
| Akkiprik et al 2009 [19]      | Turkey     | 60    | 27         | 25         | 8          | 60          | 39       | 16         | 5          |            |            |            | 0.15 |
| Buyru et al 2007 [29]         | Turkey     | 97    | 59         | 35         | 3          | 107         | 61       | 43         | 3          |            |            |            | 0.87 |
| Cherdyntseva et al 2012 [30]  | Russia     | 115   | 83         | 28         | 4          | 63          | 47       | 15         | 1          |            |            |            | 0.13 |
| Costa et al 2008 [18]         | Portugal   | 191   | 122        | 56         | 13         | 216         | 147      | 65         | 4          |            |            |            | 0.29 |
| De Vecchi et al 2008 [31]     | Italy      | 350   | 233        | 103        | 14         | 352         | 256      | 87         | 9          |            |            |            | 0.62 |
| Gaudet et al 2007 [32]        | USA (M)    | 578   | 404        | 157        | 17         | 390         | 272      | 108        | 10         |            |            |            | 0.85 |
| Gohari-Lasaki et al 2015 [24] | Iran       | 100   | 53         | 38         | 9          | 100         | 60       | 37         | 3          |            |            |            | 0.34 |
| Guleria et al 2012 [33]       | India      | 80    | 43         | 30         | 7          | 80          | 53       | 25         | 2          |            |            |            | 0.64 |
| Hao et al 2018 [34]           | Chine      | 254   | 230        | 24         | 0          | 252         | 227      | 25         | 0          |            |            |            | 0.41 |
| Hrstka et al 2009 [35]        | Island     | 117   | 81         | 32         | 4          | 108         | 81       | 24         | 3          |            |            |            | 0.46 |
| Morten et al 2019 [21]        | Australia  | 1304  | 986        | 289        | 29         | 436         | 325      | 104        | 7          |            |            |            | 0.67 |
| Pouladi et al 2014 [36]       | Iran       | 221   | 135        | 69         | 17         | 170         | 107      | 51         | 12         |            |            |            | 0.10 |
| Sharma et al 2014 [7]         | India      | 200   | 134        | 52         | 14         | 200         | 137      | 55         | 8          |            |            |            | 0.41 |
| Suspitsin et al 2003 [37]     | Russia     | 529   | 408        | 108        | 13         | 249         | 187      | 56         | 6          |            |            |            | 0.47 |
| Trifa et al 2010 [38]         | Tunisia    | 159   | 98         | 56         | 5          | 132         | 86       | 41         | 5          |            |            |            | 0.97 |
| Vymetalkova et al 2015 [39]   | Czech      | 705   | 474        | 164        | 24         | 611         | 421      | 172        | 18         |            |            |            | 0.93 |
| Wang-Gohrke et al 2002 [17]   | Germany    | 563   | 370        | 173        | 20         | 549         | 391      | 145        | 13         |            |            |            | 0.92 |
| Weston et al 1997 [40]        | USA (M)    | 99    | 60         | 36         | 3          | 185         | 127      | 54         | 4          |            |            |            | 0.52 |

M : Mixed ; N = Number

**Quantitative Analysis**

This meta-analysis showed a significant association between PIN3 16-bp duplication polymorphism and breast cancer risk in recessive (Fixed effect model (FEM): OR=1.46, 95% CI = 1.15-1.85; p = 0.002) and additive (FEM: OR = 1.11, 95% CI = 1.02-1.19; p = 0.01) models, but not in the dominant model (FEM: OR=1.07, 95% CI = 0.98-1.20; p = 0.15). Figures 2, 3, and 4, show the forest plots of OR for breast cancer in the dominant, recessive and additive models of PIN3 16-bp duplication polymorphism of the TP53, respectively.

**Sensitivity Analysis**

The stability of the results was assessed by a sensitivity analysis. We have noted a significant association between the PIN3 16-bp duplication polymorphism and the risk of breast cancer in the
recessive (Figure 3) and additive (Figure 4) models, except the dominant model (Figure 2). Furthermore, the one by one elimination of eligible studies did not influence the values of the pooled OR effect in the different genetic models.

**Sources of Heterogeneity**

After the non-inclusion of articles with HWE-deviation in controls, we noted a lack of heterogeneity in the dominant ($I^2 = 19\%, P = 0.23$), recessive ($I^2 = 0\%, P = 0.94$) and additive ($I^2 = 11\%, P = 0.32$) models between PIN3 16-bp duplication polymorphism and breast cancer risk (Figures 2, 3, and 4).

**Publication Bias**

A funnel plot was used to assess publication bias. After the elimination of studies that did not meet the inclusion criteria followed by the sensitivity analysis, no publication bias was observed in the recessive and additive models. However, a slight asymmetry was detected in the dominant model (Figure 5).

**Discussion**

In the present study, we noted a positive correlation of the PIN3 16-bp duplication polymorphism of TP53 with the histological type of breast cancer. Similar results have been found in the Iranian population by Faghani et al. who reported a correlation between invasive ductal breast cancer and the PIN3 duplication polymorphism at 16 bp [41]. Contrary to our observations, studies carried out in the Moroccan, Croatian and Czech populations have not found any link between histological types and mutations in this gene [20,27,35]. These contradictory results may be explained by the ethnic and geographic origin.

Our results show that the PIN3 16-bp duplication polymorphism is significantly associated with the risk of developing breast cancer in the Malian population. We found that heterozygous, dominant and A1A2 additive models were significantly associated with an increased risk of breast cancer. However, the results of various studies regarding the association between the PIN3 16-bp duplication of TP53 and the risk of breast cancer are contradictory. Similar to our results, Faghani et al. and Wu et al. reported that the A1A2 genotype is associated with the risk of breast cancer [41-42]. On the other hand, others studies have found no association between this genotype and the risk of breast cancer [19, 27-28]. However, we noted that the A2A2 genotype was not associated with the risk of breast cancer in our population. This observation was similar to those previously reported by in Morocco [20], in Iran [41], and Poland [43] but contradictory with the result obtained in Portugal [18]. In addition, we noted that the A2 allele was associated with the risk of breast cancer, which was consistent with the results of many authors [33, 41] but different from the results reported by others [34, 39]. The differences between studies may be explained by several factors such as sample size, race, ethnic differences, genetic background, environmental factors and heterogeneity between the studies.

The meta-analysis, which included 6,018 breast cancer patients and 4,456 controls revealed an increased-risk of breast cancer with the recessive and additive models of PIN3 16-bp duplication. Two previous
meta-analyzes, one covering 19 studies with 4479 cases and 4683 controls [42] and the other covering 9 studies with 2,715 cases and 2,595 controls [22] showed that the recessive model was associated with the risk of breast cancer. However, another meta-analysis of 6 studies with 2,018 cases and 1,748 controls revealed an inverse association [23], but the number of studies included and the sample size for this study were relatively small. Compared to our results, all these meta-analyzes found a significant genetic association between the additive model and breast cancer [22, 23, 42]. The mechanism associating A2 with breast cancer is not yet fully established, certain factors have been discussed. There is some evidence linking A2 status of differential expression of different p53 isoforms in lymphoblastoid cell lines, thereby causing alteration in mRNA [14, 44, 45]. Indeed, the influence of A2 allele on the alternative splicing of p53 protein causes an instability of the transcripts or proteins with modified functions. Many investigators have reported the existence of linkage disequilibrium between 6-bp duplication and other variants of TP53 such as codon 72 or Arg72Pro, intron 6. The codon72/intron 3 16-bp duplication of TP53 haplotype was associated with the ability to repair DNA in lymphoblastic cell lines and apoptic reduction [22, 46]. Thus, the polymorphisms of TP53 could affect the activity of p53 by triggering the process of carcinogenesis.

This study has some limitations such as small sample size, lack of hormonal receptors tests and subgroup analyzes in the meta-analysis. Another limitation is the collection of data limited to the demographic parameters and history of the disease in controls.

**Conclusions**

The present study made it possible to establish for the first time the distribution of alleles and genotypes of PIN3 16-bp duplication polymorphism of TP53 in the Malian population and to understand the relationship between this gene and the risk of breast cancer. Our results have shown that this polymorphism is not only associated with the histological type, but also is with the risk of breast cancer in Malian population. In addition, the meta-analysis carried out confirmed our findings.

**Abbreviations**

AS-PCR: Allele Specific PCR

CHU: University Hospital Center

CI: Confidence Interval

FEM: Fixed effect model

HWE: Hardy-Weinberg Equilibrium

LMICs: Low- and middle-income countries

OR: Odd ratio
Declarations

ACKNOWLEDGMENTS

Research reported in this publication was supported by the Fogarty International Center and the National Institutes of Health under Award Number D43 TW010543. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors also thank all participants in the study; the University Clinical Research Center (UCRC-Mali), Intelligence Center of Excellence Mali (ICER-Mali); Cheick Fantamady Traore, Prof. Mamadou Diakite and Dr Mamadou Coulibaly for logistical support.

Funding

This study was financially supported by the HBNU Consortium, Fogarty International Center, Global Health Fellowship Training Programs and the National Institutes of Health under Award Number D43 TW010543.

Availability of data and materials

The datasets generated and/or analyzed in this study are available from the corresponding author upon reasonable request and with the permission of FMPOS Ethics Committee.

Ethics approval and consent to participate

This study was approved by the FMPOS Ethics Committee (IRB N° 2018/63/CE/FMPOS), Université des Sciences, des Techniques et des Technologies de Bamako (USTTB), Mali. All participants accepted and signed the written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author contributions

All authors read and approved the final manuscript. Study concept and design: BD, YK, OK, JW, EN, GD, EC, SN, SD, LH, MM. Clinical data collection: MK, CBT, BK. Acquisition of genetic data: BD, YK, OK. Analysis and interpretation of data: BD, YK, OK, MM, MLK, JW, JM, EN, BJ, CBT, GD, BK, ED, SN, MI, JLH, RM, SD, LH. Drafting of the manuscript: BD with assistance from by BD, YK, OK, MM, MLK, JW, JM, EN,
BJ, CBT, GD, BK, ED, SN, MI, JLH, RM, SD, LH. Critical revision of the manuscript for important intellectual content: BD, YK, OK, MM, MLK, JW, JM, EN, BJ, CBT, GD, BK, ED, SN, MI, JLH, RM, SD, LH. Obtaining supervision: MM, LH, RM.

References

1. Fenga C. Occupational exposure and risk of breast cancer. Biomed Rep. 2016;4:282–92.
2. DeSantis CE, Bray F, Ferlay J, Lortet-Tieulent J, Anderson BO, Jemal A. International Variation in Female Breast Cancer Incidence and Mortality Rates. Cancer Epidemiol Biomarkers Prev. 2015;24:1495–506.
3. Veeraraghavan J, Ma J, Hu Y, Wang X-S. Recurrent and pathological gene fusions in breast cancer: current advances in genomic discovery and clinical implications. Breast Cancer Res Treat. 2016;158:219–32.
4. Jara L, Morales S, de Mayo T, Gonzalez-Hormazabal P, Carrasco V, Godoy R. Mutations in BRCA1, BRCA2 and other breast and ovarian cancer susceptibility genes in Central and South American populations. Biol Res. 2017;50:35.
5. Bunz F, Hwang PM, Torrance C, Waldman T, Zhang Y, Dillehay L, et al. Disruption of p53 in human cancer cells alters the responses to therapeutic agents. J Clin Invest. 1999;104:263–9.
6. Lehmann-Che J, Turpin E, Bertheau P, Espié M, de Thé H. [Exquisite sensitivity of TP53 mutant breast cancers to dose-dense chemotherapy]. Med Sci (Paris). 2007;23:1021–3.
7. Sharma S, Sambyal V, Guleria K, Manjari M, Sudan M, Uppal MS, et al. TP53 polymorphisms in sporadic North Indian breast cancer patients. Asian Pac J Cancer Prev. 2014;15:6871–9.
8. Lamb P, Crawford L. Characterization of the human p53 gene. Mol Cell Biol. 1986;6:1379–85.
9. Sommer SS, Cunningham J, McGovern RM, Saitoh S, Schroeder JJ, Wold LE, et al. Pattern of p53 gene mutations in breast cancers of women of the midwestern United States. J Natl Cancer Inst. 1992;84:246–52.
10. Blaszyk H, Hartmann A, Tamura Y, Saitoh S, Cunningham JM, McGovern RM, et al. Molecular epidemiology of breast cancers in northern and southern Japan: the frequency, clustering, and patterns of p53 gene mutations differ among these two low-risk populations. Oncogene. 1996;13:2159-66.
11. Hartmann A, Blaszyk H, Kovach JS, Sommer SS. The molecular epidemiology of p53 gene mutations in human breast cancer. Trends Genet. 1997;13:27–33.
12. Isobe M, Emanuel BS, Givol D, Oren M, Croce CM. Localization of gene for human p53 tumour antigen to band 17p13. Nature. 1986;320:84–5.
13. Zhang Z, Wang M, Wu D, Wang M, Tong N, Tian Y, et al. P53 codon 72 polymorphism contributes to breast cancer risk: a meta-analysis based on 39 case-control studies. Breast Cancer Res Treat. 2010;120:509–17.
14. Gemignani F, Moreno V, Landi S, Moullan N, Chabrier A, Gutiérrez-Enríquez S, et al. A TP53 polymorphism is associated with increased risk of colorectal cancer and with reduced levels of TP53 mRNA. Oncogene. 2004;23:1954–6.

15. Lazar V, Hazard F, Bertin F, Janin N, Bellet D, Bressac B. Simple sequencerepeat polymorphism within the p53 gene. Oncogene. 1993;8:1703-5.

16. Hillebrandt S, Streffer C, Demidchik EP, Biko J, Reiners C. Polymorphisms in the p53 gene in thyroid tumours and blood samples of children from areas in Belarus. Mutat Res. 1997;381:201–7.

17. Wang-Gohrke S, Becher H, Kreienberg R, Runnebaum IB, Chang-Claude J. Intron 3 16 bp duplication polymorphism of p53 is associated with an increased risk for breast cancer by the age of 50 years. Pharmacogenetics. 2002;12:269–72.

18. Costa S, Pinto D, Pereira D, Rodrigues H, Cameselle-Teijeiro J, Medeiros R, et al. Importance of TP53 codon 72 and intron 3 duplication 16bp polymorphisms in prediction of susceptibility on breast cancer. BMC Cancer. 2008;8:32.

19. Akkiprik M, Sonmez O, Gulluoglu BM, Caglar HB, Kaya H, Demirkalem P, et al. Analysis of p53 gene polymorphisms and protein over-expression in patients with breast cancer. Pathol Oncol Res. 2009;15:359–68.

20. Marouf C, Tazzite A, Diakité B, Jouhadi H, Benider A, Nadifi S. Association of TP53 PIN3 polymorphism with breast cancer in Moroccan population. Tumor Biol. 2014; 35: 12403-8.

21. Morten BC, Chiu S, Oldmeadow C, Lubinski J, Scott RJ, Avery-Kiejda KA. The intron 3 16 bp duplication polymorphism of p53 (rs17878362) is not associated with increased risk of developing triple-negative breast cancer. Breast Cancer Res Treat. 2019;173:727–33.

22. Hu Z, Li X, Yuan R, Ring BZ, Su L. Three common TP53 polymorphisms in susceptibility to breast cancer, evidence from meta-analysis. Breast Cancer Res Treat. 2010;120:705–14.

23. Sagne C, Marcel V, Amadou A, Hainaut P, Olivier M, Hall J. A meta-analysis of cancer risk associated with the TP53 intron 3 duplication polymorphism (rs17878362): geographic and tumor-specific effects. Cell Death Dis. 2013;4:e492.

24. Gohari-Lasaki S, Gharesouran J, Ghojazadeh M, Montazeri V, MohaddesArdebili SM. Lack of influence of TP53 Arg72Pro and 16bp duplication polymorphisms on risk of breast cancer in Iran. Asian Pac J Cancer Prev. 2015;16:2971–4.

25. Hashemi M, Amininia S, Ebrahimi M, Simforoosh N, Basiri A, Ziaee SAM, et al. Association between polymorphisms in TP53 and MDM2 genes and susceptibility to prostate cancer. Oncol Lett. 2017;13:2483–9.

26. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21:1539–58.

27. Bisof V, Salihović MP, Narancić NS, Skarić-Jurić T, Jakić-Razumović J, Janićijević B, Turek S, Rudan P. TP53 gene polymorphisms and breast cancer in Croatian women: a pilot study. Eur J Gynaecol Oncol. 2010;31(5):539-44.
28. Alawadi S, Ghareau L, Alsaleh M, Abdulaziz Z, Rafeek M, Akil N, et al. P53 gene polymorphisms and breast cancer risk in Arab women. Med Oncol. 2011;28:709–15.

29. Buyru N, Altinisik J, Demokan S, Dalay N. p53 genotypes and haplotypes associated with risk of breast cancer. Cancer Detect Prev. 2007;31:207–13.

30. Cherdyntseva NV, Denisov EV, Litviakov NV, Maksimov VN, Malinovskaya EA, Babyshkina NN et al. Crosstalk between the FGFR2 and TP53 genes in breast cancer: data from an association study and epistatic interaction analysis. DNA Cell Biol. 2012;31:306-16.

31. De Vecchi G, Verderio P, Pizzamiglio S, Manoukian S, Bernard L, Pensotti V, et al. The p53 Arg72Pro and Ins16bp polymorphisms and their haplotypes are not associated with breast cancer risk in BRCA-mutation negative familial cases. Cancer Detect Prev. 2008;32:140–3.

32. Gaudet MM, Gammon MD, Bensen JT, Sagiv SK, Shantakumar S, Teitelbaum SL, et al. Genetic variation of TP53, polycyclic aromatic hydrocarbon-related exposures, and breast cancer risk among women on Long Island, New York. Breast Cancer Res Treat. 2008;108:93-9.

33. Guleria K, Sharma S, Manjari M, Uppal MS, Singh NR, Sambyal V. p.R72P, PIN3 Ins16bp polymorphisms of TP53 and CCR5?32 in north Indian breast cancer patients. Asian Pac J Cancer Prev. 2012;13:3305-11.

34. Hao W, Xu X, Shi H, Zhang C, Chen X. No association of TP53 codon 72 and intron 3 16-bp duplication polymorphisms with breast cancer risk in Chinese Han women: new evidence from a population-based case-control investigation. Eur J Med Res. 2018;23:47.

35. Hrstka R, Beranek M, Klocova K, Nenutil R, Vojtesek B. Intronic polymorphisms in TP53 indicate lymph node metastasis in breast cancer. Oncol Rep. 2009;22:1205-11.

36. Pouladi N, Kouhsari SM, Feizi MH, Dehghan R, Azarfam P, Farajzadeh D. Lack of association of intron 3 16 bp polymorphism of TP53 with breast cancer among Iranian-Azeri patients. Asian Pac J Cancer Prev. 2014;15:2631-4.

37. Suspitsin EN, Buslov KG, Grigoriev MY, Ishutkina JG, Ulibina JM, Gorodinskaya VM, et al. Evidence against involvement of p53 polymorphism in breast cancer predisposition. Int J Cancer. 2003;103:431–3.

38. Trifa F, Karray-Chouayekh S, Mabrouk I, Baccouche S, Khabir A, Sellami-Boudawara T, et al. Haplotype analysis of p53 polymorphisms: Arg72Pro, Ins16bp and G13964C in Tunisian patients with familial or sporadic breast cancer. Cancer Epidemiol. 2010;34:184–8.

39. Vymetalkova V, Soucek P, Kunicka T, Jiraskova K, Brynychova V, Pardini B et al. Genotype and Haplotype Analyses of TP53 Gene in Breast Cancer Patients: Association with Risk and Clinical Outcomes. PLoS One. 2015;30;10:e0134463.

40. Weston A, Pan CF, Ksieski HB, Wallenstein S, Berkowitz GS, Tartter PI, et al. p53 haplotype determination inbreast cancer. Cancer Epidemiol Biomarkers Prev. 1997;6:105-12.

41. Faghani M, Ghasemi FM, Nikhbakht M, Salehi M. TP53 PIN3 polymorphism associated with breast cancer risk in Iranian women. Indian J Cancer. 2011;48:298–302.
42. Wu D, Zhang Z, Chu H, Xu M, Xue Y, Zhu H, et al. Intron 3 sixteen base pairs duplication polymorphism of p53 contributes to breast cancer susceptibility: evidence from meta-analysis. PLoS One. 2013;8:e61662.

43. Jakubowska A, Gronwald J, Menkiszak J, Górski B, Huzarski T, Byrski T, et al. BRCA1-associated breast and ovarian cancer risks in Poland: no association with commonly studied polymorphisms. Breast Cancer Res Treat. 2010;119:201–11.

44. Courtois S, Verhaegh G, North S, Luciani M-G, Lassus P, Hibner U, et al. DeltaN-p53, a natural isoform of p53 lacking the first transactivation domain, counteracts growth suppression by wild-type p53. Oncogene. 2002;21:6722–8.

45. Ghosh A, Stewart D, Matlashewski G. Regulation of human p53 activity and cell localization by alternative splicing. Mol Cell Biol. 2004;24:7987–97.

46. Wu X, Zhao H, Amos CI, Shete S, Makan N, Hong WK, et al. p53 genotypes and haplotypes associated with lung cancer susceptibility and ethnicity. J Natl Cancer Inst. 2002;94:681-690.

Figures
Initial search
N= 57

Meet PIN316-bp and case-control inclusion criteria
N= 27

No PIN316-bp or not case-control study and excluded
N= 30

Meet Hardy-Weinberg Equilibrium (HWE) in controls criteria
N= 21

Controls deviating HWE and excluded N= 6

Meet no influence on overall OR and p value criteria
N= 19

Value with overall OR and p-value influenced and excluded
N= 2

Included in meta-analysis
N= 19

Figure 1
Flow chart of meta-analysis for exclusion/inclusion of studies
Figure 2

Forest plots of the relationship between PIN3 16-bp duplication polymorphism of the TP53 and breast cancer in the dominant model. The black diamond denotes the pooled OR; black squares indicate the OR in each study with square sizes inversely proportional to the standard error of the OR; and horizontal lines represent the 95% CI.

Figure 3

Forest plots of the relationship between PIN3 16-bp duplication polymorphism of the TP53 and breast cancer in the dominant model. The black diamond denotes the pooled OR; black squares indicate the OR in each study with square sizes inversely proportional to the standard error of the OR; and horizontal lines represent the 95% CI.
Forest plots of the relationship between PIN3 16-bp duplication polymorphism of the TP53 and breast cancer in the recessive model. The black diamond denotes the pooled OR; black squares indicate the OR in each study with square sizes inversely proportional to the standard error of the OR; and horizontal lines represent the 95% CI.

### Figure 4

Forest plots of the relationship between PIN3 16-bp duplication polymorphism of the TP53 and breast cancer in the additive model. The black diamond denotes the pooled OR; black squares indicate the OR in each study with square sizes inversely proportional to the standard error of the OR; and horizontal lines represent the 95% CI.

### Figure 5

Funnel plots of (a) dominant, (b) recessive and (c) additive models precision by OR