TP53 p.Arg72Pro polymorphism and Breast Cancer Risk: A meta-analysis of case-control studies

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Brehima Diakite
Universite des Sciences des Techniques et des Technologies de Bamako

br.diakite@yahoo.fr Corresponding Author
ORCiD: https://orcid.org/0000-0001-8296-5292

Yaya Kassogue
University des Sciences, techniques et Technologies de Bamako

Guimogo Dolo
Faculty of Medicine and Odontostomatology/ Universite des Sciences, Techniques et Technologies de Bamako

Jun Wang
Northwestern University

Erin Neuschler
University of Illinois Chicago

Oumar Kassogue
Universite des Sciences, Techniques et Technologies de Bamako

Mamadou Keita
CHU du Point G

Cheick Bougari Traore
Faculty of Medicine and Odontostomatology/Universite des Sciences, Techniques et Technologies de Bamako

Bakarou Kamate
Universite des Sciences, Techniques et Technologies de Bamako

Etienne Dembele
Northwestern University Department of Biomedical Engineering

Nadifi Sellama
Universite Hassan II Casablanca

Robert Murphy
Northwestern University Department of Biomedical Engineering

Seydou Doumbia
Universite des Sciences, techniques et Technologies de Bamako

Lifang Hou
Northwestern University Department of Biomedical Engineering

Mamoudou Maiga
Northwestern University Department of Biomedical Engineering

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Abstract
Background: The effect of the p.Arg72Pro variant of the P53 gene on the risk of development of breast cancer remains variable in populations. However, the use of strategies such as pooling age-matched controls with disease cases may provide a solid meta-analysis. Our goal was to perform a meta-analysis in order to assess the association of p.Arg72Pro variant of P53 gene with breast cancer risk.

Methods: Databases such as PubMed, Genetics Medical Literature, Harvard University Library, Web of Science and Genesis Library were used to search articles. Age-matched case-control studies on breast cancer that have evaluated the genotype frequencies of the p.Arg72Pro of P53 gene were selected. The fixed and random effects (Mantel-Haenszel) were calculated using pooled odds ratio of 95% CI to determine the risk of disease. Inconsistency was calculated to determine heterogeneity among the studies. The publication bias was estimated using the funnel plot.

Results: Twenty-one publications with cases age-matched controls including 7841 disease cases and 8876 controls were evaluated in this meta-analysis. Overall, our results suggested that p.Arg72ProP53 was associated with a risk for breast cancer for the dominant model (OR = 1.09, 95% CI = 1.02-1.16; P = 0.01) and the additive model (OR = 1.09, 95% CI = 1.01-1.17; P = 0.03), but not in the recessive model (OR = 1.07, 95% CI = 0.97-1.16; P = 0.19). According to the ethnic group, allele Pro has been associated with breast cancer risk in Europeans for the dominant and additive models.

Conclusions: This meta-analysis found a significant association between p.Arg72Pro in the P53 gene and the risk of breast cancer. Individuals carrying at least one Pro allele of the P53 gene are more likely to have breast cancer with dominant and additive models than individuals harboring the Arg allele.

Background
Breast cancer, is a multifactorial disease with a strong genetic component; it is the leading cause of death among women around the world, representing a major public health problem [1]. According to the International Agency for Research on Cancer/World Health Organization, 2.09 million new cases of breast cancer were detected worldwide in 2018 [2] against 1.38 million cases in 2008[3]. The
incidence of breast cancer differs among different populations in the world [2]. In recent decades, spectacular progress has been made in understanding the molecular genetics of breast cancer pathology. In addition to the direct involvement of genetic predisposition genes, other genes participating in cell division regulation are also implicated in the occurrence of breast cancer [4-5], such as P53 gene, a tumor suppressor gene. While the role of P53 gene is not fully elucidated yet, it is recognized that P53 plays a key role in the regulation of cell proliferation and apoptosis. The P53 protein is essential to maintain the integrity of the cell and its components. In human cancers, mutated P53 produces abnormal proteins that alter or inhibit transcriptional regulation [6]. Consequently, a cell cannot respond to stress, and the cell cycle as well as apoptosis are inhibited. Genomically, inactivation or mutation of P53 gene would be responsible for a linkage disequilibrium in the DNA sequence leading to genomic instability [7]. These abnormalities of P53 gene protein associated with chromosomal aberrations could induce the development of breast and ovarian cancer [8]. This gene is known to be the most frequently mutated in human cancers [9]. The gene is located on chromosome 17p13 and contains 11 exons. Several polymorphisms have been identified, but the most widely studied variant is the substitution of Arginine by Proline at position 72. p.Arg72Pro variant is located in exon 4, and has been shown to be associated with many pathologies including cancer [10-11]. Although many association studies on candidate genes have investigated the relationship between the p.Arg72Pro of the P53 gene and the risk of breast cancer, the reports from these studies remain contradictory. Some studies have shown that p.Arg72Pro is associated with the risk of breast cancer, while others found no associations. The studies carried out by Menzel et al. 2004 [12] and Akkiprik et al. 2009 [13] have shown a link between p.Arg72Pro and breast cancer risk. However, another age-unmatched case-control study in the similar population concluded that p.Arg72Pro was not associated with the risk of breast cancer [14]. This inconsistency in the relationship between p.Arg72Pro of the P53 gene and breast cancer risk may be explained by a very high heterogeneity in the frequency of mutations. This heterogeneity is likely related to the geographical origin of patients, the ethnicity [15-17] and the age-unmatched controls with patients’ group of the same population. In view of all these observations, the present meta-analysis will include
only age-matched case-control studies, to qualitatively assess the effect of p.Arg72Pro on the risk of breast cancer.

Methods

Literature search
The Pubmed Genetics Medical Literature Database, the Harvard University Library, and the Web of Science and Genesis Library were used to identify available articles published in English. The keywords "P53", "p.Arg72Pro" and "polymorphism" or "mutation" or "gene" and "breast cancer" cited in the genetic association studies were used to detect and select scientific manuscripts in these databases. We also reviewed references cited in these studies to identify additional articles that were not identified by our research in the databases.

Inclusion criteria
The inclusion criteria were: (1) published case-control studies as an original article to evaluate the association between p.Arg72Pro of the P53 gene and risk of breast cancer, (2) full manuscript available, (3) case-control study with age-matched, (4) distribution of genotype respecting Hardy-Weinberg equilibrium (HWE) in controls, (5) availability of the three genotypic frequencies (Arg/Arg, Arg/Pro and Pro/Pro) in the case and control groups. (6) Three investigators independently evaluated each study to determine eligibility.

Data extraction
The data were collected by an investigator and verified by a second investigator to reach consensus on all points. First author, year of publication, country, ethnicity of study population, sample size, age-matched, distribution of genotype and alleles, as well as the recalculation of HWE in controls were extracted from the eligible studies. A third reviewer made a contradictory assessment to reconcile the assumptions. The data of controls evaluated with p.Arg72Pro variant were included in this meta-analysis.

Statistical analysis
Chi² analysis with a significance level of P < 0.05 was used to evaluate whether p.Arg72Pro polymorphism distribution of the P53 gene in controls fits Hardy-Weinberg equilibrium (HWE). The
association between the p.Arg72Pro and the risk of breast cancer was evaluated by the Odd ratio (OR) of 95% CI. We evaluated the association strength of Arg72Pro polymorphism of P53 gene was made with the genetic models: dominant (Pro/Pro + Arg/Pro vs. Arg/Arg), recessive (Pro/Pro vs. Arg/Arg + Arg/Pro) and additive (Pro vs. Arg). The hypothesis of heterogeneity among the studies was assessed by $I^2$ statistical test [18-19]. If $I^2 > 50\%$ (presence of heterogeneity), the random effects model was used to calculate the overall OR, otherwise (lack of heterogeneity), the fixed effects method has been used. We also have examined the funnel plot to determine publication bias [20]. All statistical analyses were performed with Review Manager Software version 5.1.

Results
In the light of our results, 81 case-control studies from the literature search (Fig. 1) that investigated the association of p.Arg72Pro of P53 gene in the context of breast cancer were included, of which only 55 studies had a genotype distribution of control population that met Hardy-Weinberg equilibrium, and 21 out of the 55 studies have cases age-matched controls (Table 1). Among these studies, the participants of 9 studies were Europeans [12-14, 21-26], 9 studies were Asians [27-35], 2 were Americans [36, 37]. Genotype distribution of the control population that met Hardy-Weinberg equilibrium was a minimum requirement for studies to be included.

Figure 1. Flow diagram of the studies evaluated for meta-analysis

Table 1. Genotype distribution of TP53 p.Arg72Pro polymorphism in breast cancer cases and age-matched controls in studies included.

The results of combined analyses for p.Arg72Pro TP53 gene were showed in Table 2.

A total of 29 cases (N=7841) and age-matched control (N=8876) studies in 81 manuscript published were included in this meta-analysis. The sample sizes (cases/controls) of the Europeans, Asians, Americans and African population studies were 2342/2318, 2244/2354, 3130/4078 and 125/126 respectively. Overall, a significant association between p.Arg72Pro of P53 gene and the risk for breast cancer was observed in the dominant model [OR (FE) = 1.09, 95% CI = 1.02-1.16; P = 0.01] and additive model [OR (RE) = 1.09, 95% CI = 1.01-1.17; P = 0.03], but not in the recessive model [OR (FE) = 1.07, 95% CI = 0.97-1.16; P = 0.19]. When subgroup analyses were conducted according to
participant ethnicities and countries, except the recessive model [OR (FE)= 1.18, 95% CI = 0.96-1.44; P= 0.12], p. Arg72Pro was associated with breast cancer risk with the dominant and additive models in Europeans. No positive association was observed with the different models in Asian and American populations. The only eligible African study has shown an increased risk of breast cancer in recessive (OR = 2.14, 95% CI = 1.08-4.23; P= 0.03) and additive (OR = 1.49, 95% CI = 1.03-2.16; P= 0.03) models (Table2).

**Tableau 2.** Distribution of p.Arg72Pro TP53 gene according to the genetic models

Overall, after elimination of studies deviating from Hardy-Weinberg equilibrium in controls and no evidence of heterogeneity (I^2> 50%) was found with the dataset analyzed in the recessive and dominant models. However, a modest heterogeneity was observed between the p.Arg72Pro variant and the risk of breast cancer in the combined analyses and Asians for the additive model (Table 2). In addition, we compared the pooled OR of the fixed and random effects, and found no statistically significant difference between the two effects, which supports strongly the consistency of the present study’s data. To maintain the stability of the meta-analysis after the non-inclusion of deviant studies of HWE and sensitivity analysis, we evaluated the influence of each study on pooled OR. After the exclusion of studies [39-42], no study has shown a significant influence of the pooled OR effect in each of the different genetic models (Table 2).

The publication bias has been evaluated using the funnel plot. After excluding studies that deviated from the Hardy-Weinberg equilibrium in controls and the studies influencing the Odd ratio values, no significant publication bias was found in dominant, recessive and additive models (Figure2).

**Figure 2.** Funnel plots of dominant (a), recessive (b) and additive (c) models precision by OR

**Discussion**

Breast cancer is a multifactorial disease and its occurrence depends on the synergistic action of clinical, biological and environment factors and mechanisms [43-44]. In addition to these risk factors, the role of specific genes in the pathology of breast cancer is increasingly evident.

The protein and the TP53 gene have been widely studied for their associations with cancers, especially breast cancer, because they play a key role in DNA repair, cell cycle control and apoptosis.
The P53 gene encodes a transcription factor that binds to DNA and promotes the expression of genes that would repair cellular damage. Therefore, P53 is a tumor suppressor that sounds the alarm when DNA damage prevents the cell from turning into a cancer cell, or even inducing cell death. In the presence of a mutation, P53 gene can no longer repair the damaged DNA, which will lead to appearance of the malignant cells responsible for tumorigenesis [46-47]. Several functional genetic variants p.Arg72Pro, p.Pro47Ser, 16-bp-Insertion allele) have been described and speculated to be associated with the risk of breast cancer incidence [13, 46-49]. Although numerous epidemiological studies have been conducted to assess the role of SNP TP53 in the risk of breast cancer in different populations. However, these results are controversial. The meta-analysis can be an adequate tool to detect the effect of a gene in diseases with a great power of confidence. Our meta-analysis evaluated the association between the variant p.Arg72Pro of the tumor suppressor P53 gene and breast cancer with eligibility criteria of case-control studies that had age-matched controls in HWE. In this meta-analysis, we found that the Pro allele of p53 gene was associated with risk of breast cancer risk for Europeans and Africans when compared to Arg allele. This finding is consistent in part with a previous meta-analysis which investigated breast cancer risk in 41 cases unmatched control studies [50], but discordant with the finding reported by Hou et al 2013 [51], Zhuo et al [52] and Hao et al [53]. The difference between these results can be explained by the presence of heterogeneity between studies, and the mixture studies with age-matched and/or unmatched controls in their analysis. This heterogeneity may be due to the difference in ethnicity. The effects of ethnicity can be explained by several factors including allelic heterogeneity between populations, ie the same locus, but different causal variants can influence the risk of cancer from one population to another or gene-gene and gene-environment interaction and the variation of linkage disequilibrium among population [54, 55, 56]. However, in the previous meta-analyzes, the inclusion criteria of studies were not robust enough, the case with age-matched control studies and the non-deviation in HWE of the distribution of p.Arg72Pro in controls were not taken into account in the analyzes. Several studies (cases and controls, and case with age-matched controls) between p.Arg72Pro of P53 gene and the risk of breast cancer have been reported, which improved the power of the meta-analysis of this genetic variant.
The analysis of subtypes studies based on the case and age matched controls and ethnicity being possible now. So, to have a more precise estimate of genetic associations, we have updated this meta-analysis. In addition, the meta-analyzes of Goncalves et al. 2013 [50], He et al. 2011 [57] and Ma et al. 2011 [58] showed that the Arg allele of the P53 gene was not associated with the risk of breast cancer, which is consistent with our findings. The literature is composed of contradictory conclusions regarding the association of Arg72Pro P53 gene with breast cancer risk, but most of the previous meta-analyses focused on the presence or absence of the wild-type (Arg) allele in these genetic models: dominant (Arg/Arg+Pro/Arg vs. Pro/Pro), recessive (Arg/Arg vs. Arg/Pro+Pro/Pro) and additive (Arg vs. Pro) [57-58]. However, we have found some bias in certain studies regarding the criteria for inclusion of scientific articles, which may have influenced the interpretation of these meta-analyzes. This bias existed in mostly studies whose distribution of Arg/Arg, Arg/Pro and Pro/Pro genotypes in controls was not in HWE [59-72]. We also performed an in-depth sensitivity analysis by removing each single study from the pooled data and the results showed that there was no influence of the individual data on the overall results. In addition, we also calculated the overall combined OR on the additive model (Pro vs Arg) with and without the four studies which influenced the values of the Odds ratio [39-42], and in both cases, we found that the Pro allele was associated with a high risk of developing breast cancer. The major advantage of this meta-analysis was the inclusion of a large number of samples including very selective criteria, in order to identify a statistically significant association in one of the genetic models. However, several limitations need to be highlighted. Case age-matched control studies were rare in some ethnic groups, only one African study met the inclusion criteria and two studies in Americans.

Conclusions
In the light of this meta-analysis, individuals carrying at least one Pro allele of the P53 gene are more likely to have breast cancer with dominant and additive models than individuals carrying the Arg wild-type allele. Our study further reinforced and confirmed the hypothesis that the P53 gene is usually mutated in about half of breast cancer cases. For the stability and homogeneity of results from meta-analysis, future similar studies must consider criteria for selecting articles such as the HWE.
agreement and controls age-matched cases studies. Future studies should also consider comparing different ethnic groups.

Abbreviations
Arg: Arginine; CI: Confidence interval, CIRC: International Center for Research on Cancer; Fig.: Figure; HWE: Hady-Weinberg Equilibrium; $I^2$: Inconsistency; N: Number; OR: Odd ratio; P: P value Pro: Proline.

Declarations
Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable.

Availability of data and materials
The dataset analyzed for this study is available from the table 1.

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
All authors read and approved the final manuscript. Study concept and design : BD, YK, GD, JW, EN, OK, MK, CBT, BK, ED, SN, SD, LH, MM. Acquisition of data : BD, YK. Analysis and interpretation of data : BD, YK, MM. Drafting of the manuscript : BD with assistance from by YK, MM. Critical revision of the manuscript for important intellectual content : JW, EN, SN, GD, SD, LH. Obtaining supervision : RM.

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Tables

**Table 1.** Genotype distribution of TP53 p.Arg72Pro polymorphism in breast cancer cases and age-matched controls in studies included.

| Authors                  | N    | Arg/Arg | Arg/Pro | Pro/Pro | N    |
|--------------------------|------|---------|---------|---------|------|
| Akkiprik et al 2009 [13] | 95   | 25      | 50      | 20      | 10   |
| Alshatwi et al 2012 [27] | 100  | 22      | 52      | 26      | 10   |
| Ayoubi et al 2018 [38]   | 125  | 55      | 42      | 28      | 12   |
| Buyru et al 2003 [21]    | 115  | 64      | 39      | 12      | 6    |
| Cherdyntseva et al 2012 [22] | 388 | 184    | 162    | 42      | 27   |
| Costa et al 2008 [23]    | 175  | 98      | 61      | 16      | 21   |
| Cox et al 2007 [36]      | 1477 | 804     | 569     | 104     | 22   |
| Denisov et al 2009 [24]  | 297  | 148     | 124     | 25      | 27   |
| Ebner et al 2010 [25]    | 263  | 138     | 108     | 17      | 25   |
| Hossain et al 2016 [28]  | 125  | 54      | 42      | 29      | 12   |
| Isakova et al 2017 [29]  | 117  | 57      | 50      | 10      | 10   |
| Katiyar et al 2003 [30]  | 77   | 20      | 51      | 6       | 4    |
| Krivokuca et al 2014 [14] | 155 | 87      | 58      | 10      | 11   |
| Li et al 2002 [31]       | 28   | 11      | 10      | 7       | 5    |
| Ma et al 2006 [32]       | 404  | 149     | 178     | 77      | 47   |
| Menzel et al 2004 [12]   | 302  | 158     | 114     | 30      | 47   |
| Sharma et al 2014 [33]   | 200  | 47      | 103     | 50      | 20   |
| Song et al 2009 [34]     | 1110 | 341     | 547     | 222     | 10   |
| Sprague et al 2007 [37]  | 1653 | 909     | 644     | 100     | 18   |
| Wang-Gohrke et al 2002 [26] | 552 | 282    | 221    | 49      | 54   |
| Zhang et al 2007 [35]    | 83   | 21      | 45      | 17      | 16   |

N: Number, Arg/Arg: wild-type, Arg/Pro: heterozygous, Pro/Pro: mutated homozygous, HWE: Hardy-
Table 2. Distribution of p.Arg72Pro TP53 gene according to the genetic models

| Group    | N  | Sample size | Genetic Models | Overall OR (95% CI) | P-value | H  |
|----------|----|-------------|----------------|---------------------|---------|----|
| All      | 21 | 7841/8876   | Recessive      | 1.07 (0.97-1.16) FE | 0.19    | 3i |
|          |    |             | Dominant       | 1.09 (1.02-1.16) FE | 0.01    | 3' |
|          |    |             | Additive       | 1.09 (1.01-1.17) FE | 0.03    | 4' |
| European | 9  | 2342/2318   | Recessive      | 1.18 (0.96-1.44) FE | 0.12    | 0' |
|          |    |             | Dominant       | 1.16 (1.04-1.31) FE | 0.01    | 2' |
|          |    |             | Additive       | 1.13 (1.03-1.24) FE | 0.007   | 3' |
| Asian    | 9  | 2244/2354   | Dominant       | 1.05 (0.82-1.36) FE | 0.88    | 4' |
|          |    |             | Recessive      | 1.09 (0.89-1.33) FE | 0.33    | 4' |
|          |    |             | Additive       | 1.03 (0.95-1.12) RE | 0.46    | 5' |
| American | 2  | 3130/4078   | Recessive      | 1.02 (0.85-1.23) FE | 0.83    | 6' |
|          |    |             | Dominant       | 1.04 (0.95-1.15) FE | 0.38    | 0' |
|          |    |             | Additive       | 1.03 (0.96-1.11) FE | 0.42    | 4' |
| African  | 1  | 125/126     | Recessive      | 2.14 (1.08-4.23)   | 0.03    | -  |
|          |    |             | Dominant       | 1.36 (0.83-2.23)   | 0.23    | -  |
|          |    |             | Additive       | 1.49 (1.03-2.16)   | 0.23    | -  |

*: Significant, P: p value OR, p': p value heterogeneity; I^2: Inconsistency; dominant: Pro/Pro + Arg/Pro vs. Arg/Arg; recessive: Pro/Pro vs. Arg/Arg+Arg/Pro; additive: Pro vs. Arg; Phet: P value of Heterogeneity

Figures
Figure 1
Flow diagram of the studies evaluated for meta-analysis
Figure 2

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

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