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Arg Allele of *P53* Gene Codon72 is a Risk Factor for Breast Cancer in Senegalese Women

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Abstract

The proline rich domain of the *p53* tumor suppressor protein is necessary for the induction of apoptosis. A common polymorphism, Proline-72-Arginine, alters structural and biological properties of *p53*. The Arginine allele has been suggested as a risk factor for breast cancer. We investigate by association and segregation studies, the role of Arg72 allele as a risk factor in sporadic and familial breast cancer in Senegalese women. Eighty patients diagnosed with breast cancer followed up at the Curie Institute in Dakar, and eighty-five healthy controls without known cancer, were recruited after informed consent. For each individual, DNA was extracted from whole blood and codon 72 polymorphism genotyped by PCR-RFLP. Our results showed an increased risk for patients carrying Arg/Arg and Arg/Pro genotypes compared to those carrying Pro/Pro (p<0.03). Similarly, the Arg allele was associated with an increased risk (p<0.03). The codon 72 of *p53* might be involved in susceptibility to breast cancer.

Keywords: Breast cancer; *p53* gene; Susceptibility

Introduction

Breast cancer is becoming the top cancer in women both in the developed and the developing world. The incidence of breast cancer is increasing in the later countries due to increase life expectancy, urbanization and adoption of western lifestyles [1]. Although many risk factors for the development of breast cancer have been identified, the molecular mechanisms related to breast carcinogenesis remains unclear [2,3]. Breast cancer seems to be the result of cumulative alterations of oncogenes and/or tumor suppressor genes that lead to clonal outgrowth of progressively malignant cells [4,5]. Among the tumor suppressor genes, the *p53* gene, located on chromosome band 17p13.1, is one of the most commonly mutated genes in all types of human cancers [6]. The *p53* gene encodes a multi-functional transcription factor that participates in cell-cycle control, apoptosis, genomic stability and DNA repair [7,8]. These activities are mediated by direct binding of the *p53* tetramer to specific target sequence motifs in promoters of downstream effector genes, and by interactions between *p53* and other cellular proteins [9,10]. One of the regions of *p53* that is believed to be required for its ability to induce apoptosis is the Proline-rich domain [11]. Interestingly, within this domain, there is a common polymorphism at position 72 encoding either an Arginine or a Proline residue [12]. The replacement of Proline by Arginine abolishes one of the five PXXP motifs, which alters structural and biological properties of *p53* [13]. The Pro72 allele appears to induce a higher level of G1 arrest than the Arg72 [14].

It is therefore temping to think that Arg72 allele could be a potential genetic risk factor for cancer. Several studies highlighted an association between codon 72 of *p53* polymorphism and risk of breast cancer; however this hypothetic association remains controversial [15-18].

The purpose of this study was to investigate the role of the Arg72 allele as a risk factor for sporadic and familial breast cancer in Senegalese women. We also analyzed correlation between the genotype distribution and the clinical and prognostic features in sporadic breast cancer.

Material and Methods

Study population

This study includes 80 patients diagnosed with sporadic breast cancer and 85 healthy controls without known cancer. Patients were recruited between December 2014 and September 2015 at the Institute Curie of Aristide-Le-Dantec hospital in Dakar. Histological diagnosis of breast cancer was confirmed in each case. For familial breast cancer two families FY and AB with history of breast cancer were recruited. Proband FY had aggressive early onset breast cancer at age 25 with tumor stage T4bN2M0. Proband AB had triple negative bilateral breast cancer associated with ovarian cancer diagnosed at age 50. She and her twin sister died from breast cancer.

Control subjects were enrolled at the Laboratory of Biology of Le Dantec Hospital and were confirmed as free from breast cancer by medical examination. Written informed consents were obtained from all cases and controls.

Methods

For each case and control DNA was extracted with Qiagen Kits from peripheral blood samples. *p53* codon72 genotypes were determined using PCR-RFLP. PCR amplification of exon 4 of *p53* gene was conducted with a specific primer pair (*p53*F 5'TCC CCC TTG

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CCG TCC CAA3'; *p53*R 5'CGT GCA AGT CAC AGA CTT3'). Each PCR reaction mixture (25 µl) contained 0.5 µM of each primer, 2U of GoTaq and 2µl of genomic DNA.

PCR reactions were carried out in a thermocycler with an initial denaturation at 94°C for 5 min followed by 35 step cycles of amplification at 94°C for 30s, 58°C for 30s, 72°C for 30s and final extension at 72°C for 5 min.

Genotyping of codon72 from PCR products was performed with the restriction enzyme BstUI which specifically recognizes the 5'CGCG3' sequence and cuts the PCR product thus generating two fragments.

When BstUI restriction site is present (Arginine allele), the 279 pb fragment is digested into two fragments of respectively 160 and 119 pb while no digestion is observed for the Proline allele. Within each group, Sanger sequencing of the PCR products confirmed genotypes.

Statistical analysis

Data regarding age, tumor type, tumor stage, and tumor grade were recorded from clinical reports. The association between the *p53* codon 72 polymorphisms and breast cancer risk was estimated by Odds ratio (OR) with 95% Confidential Interval (CI). The association between [*p53*] ^Arg-72 Propolymorphism and clinical and pathological characteristics of breast cancer was assessed using X2 test of independence. A p-value <0.05 was considered as significant. All analyses were done using Statistical Package for Social Sciences software 20.0. For the 2 families with familial breast cancer, pedigrees were drawn using Progeny software.

Results

In the case/control association study, the mean age was 47.59 years ±13.85 years for female patients with sporadic breast cancer while it was 40 years for the controls with a standard deviation of 15.53 years. Mean age difference was not significant between patients and controls (*p*>5%).

Tumor histology shows that 83.75% of patients had infiltrative ductal carcinoma, 2.5% medullary carcinoma and 13.75% had other types of carcinoma. In terms of tumor-node- metastasis (TNM) clinical staging, 3 patients (3.75%) were in stage 2, 60 patients (75%) in stage 3, 7 patients (8.75%) in stage 4. For 10 patients (12.5%) stage was not established. Tumor histological grading revealed that 94.5% of patients had high-grade tumors (Grade II and III).

PCR amplification of exon 4 of the *p53* gene generated a 279 bp DNA fragment at the expected size. After digestion of the PCR product by BstUI enzyme and separation by electrophoresis, two fragments of 119 bp and 160 bp respectively were observed for Arg/Arg homozygous individuals. For Pro/Pro homozygous individuals, one 279 bp fragment was observed. Heterozygous individuals Arg/Pro showed three fragments with respective sizes 119 bp, 160 bp and 279 bp (Figure 1).

Genotypes and alleles distribution of codon72 polymorphism in patients and controls are summarized in Table 1. Case/Controls association study highlights an increased risk of breast cancer for codon72 Arg/Arg genotype (OR: 2.26; IC: 0.93 – 5.53), compared with the Pro/Pro genotype. This association was close to the threshold of significance (p=0.06). We combined the *p53* Arg/Arg and Arg/Pro genotypes into one group for subsequent analysis. We found an increased risk for patients carrying Arg/Arg or Arg/Pro genotypes compared to those carrying Pro/Pro genotype (OR: 1.96 ; IC: 1.03 – 3.70; p=0.03). Similarly, the Arg72 allele was also associated with an increased risk of developing breast cancer (OR: 1.62; IC: 1.03 – 2.53; p=0.03).

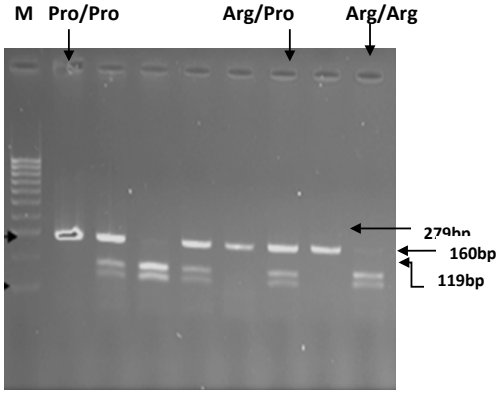

Figure 1: Electrophoresis profiles of p53 codon72 polymorphism genotypes in 1.5% agarose gel M: 100 bp DNA ladder.

Variables	Cases (N=80)		Controls (N=85)				
	n	%	n	%	*p*	or	95% IC
Genotypes							
Pro/Pro	25	31.25	40	47.06	----	1.00	Ref
Arg/Arg	17	21.25	12	14.11	0.06	2.26	0.93 – 5.53
Pro/Arg	38	47.50	33	38.82	0.07	1.84	0.93 – 3.65
Arg/Arg+Pro/Arg	55	68.75	45	52.93	0.03	1.96	1.03 --3.70
Alleles							
Pro	88	55.00	113	66.47	----	1.00	Ref
Arg	72	45.00	57	33.53	0.03	1,62	1.03 – 2.53

Table 1: Genotypes and alleles frequencies of Codon72 among cases and controls and breast cancer associated risk.

These results obtained in sporadic breast cancer have been confirmed in familial breast cancer study. We found that the Arg72 allele co-segregated with breast cancer in family FY and family AB (Figures 2A and 2B respectively). Healthy individuals from each family also have the Arg72 allele in heterozygous or homozygous status.

Relationship between codon-72 genotypes and prognostic characteristics of sporadic breast cancers tumors are shown in Table 2. Analysis of breast cancer tumor stage and histological grade showed that Arg/Arg and Arg/Pro genotypes frequencies were higher in breast cancer patients with advance tumor stage (Stages 3 and 4) and high histological grade (Grade II and III) compared to Pro/Pro genotype. But these observations did not reach statistical significance.

Discussion

Cancer is among the leading causes of death worldwide. Breast cancer is the most frequent gynecologic cancer worldwide since incidence is growing up in less developed countries [1]. Cancer care requires too many resources that are a tough challenge for less developed countries. Therefore, cancer prevention efforts must be a priority particularly in Sub Saharan Africa [19].

The epidemiology of breast cancer in these countries is characterized by younger age at diagnosis (<55 years), triple negative tumors, advanced disease stages and poor prognosis [19]. These characteristics have been found in our patients where mean age at diagnosis was 47.59 years, tumors stages 3 and 4 and poor prognosis grade III were mostly

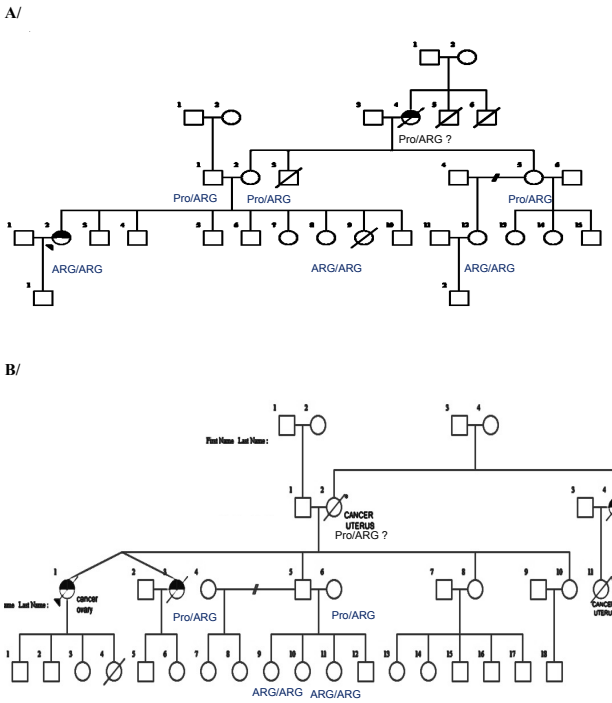

Figure 2: p53 Arg72 allele segregation in hereditary breast cancer A: Family FY; B: Family AB.

Variables	Genotypes			P
	Pro/Pro	Arg/Pro	Arg/Arg	
Characteristics				
Tumor Clinical Stage	**n (%)**	**n (%)**	**n (%)**	
2	0 (0.0)	3 (100)	0 (0.0)	0.3
3	22 (36.7)	27 (45.0)	11 (18.3)	
4	1 (14.3)	4 (57.1)	2 (28.6)	
Tumor grade	**n (%)**	**n (%)**	**n (%)**	
I	1 (25.0)	2 (50.0)	1 (25.0)	0.2
II	11 (22.9)	26 (54.2)	11 (22.9)	
III	4 (36.4)	6 (54.5)	1 (9.1)	

Table 2: Association study between Codon72 genotypes and prognostic characteristics of breast cancer tumors.

represented (95.7%) and (94.5%) respectively. To understand these specific epidemiologic features of breast cancer in African continent, country wide research programs must be conduct.

Among the potential explanations of this specific epidemiology are genetic factors. A number of case-control studies have been conducted over last decades, to investigate the association between genetic factors and breast cancer risk. Polymorphisms of the tumor suppressor gene *p53* which is involved in cancer genesis have been associated with breast cancer risk in several populations. The *p53* codon72 polymorphism (Pro72Arg) of *p53* gene has received considerable attention in the last few years [20].

In this study we report the Arg72 allele of *p53* as a risk factor for sporadic and hereditary breast cancer. Similar results have been obtained through several studies. Andrea and coworkers studied Brazilian women with breast cancer and found that Arg72 allele was significantly associated with an increased risk of breast cancer development [21]. Moreover, Abber Al-Qasem et al., report that the Arg/Arg genotype was associated with risk and early onset of breast cancer among Saudi women [22,23]. Eltahir and coworkers in Sudan reported that breast carcinoma patients most prominently showed excess of homozygous Arg genotype as compared to controls with an Odd ratio (OR) of 19.44, 95% CI: 6.6-78.3, P<0.0001) (24). Ohayan et al. also reported a similar result in Jewish patients with family history of breast cancer [22]. This is in concordance with the Arg allele segregation with breast cancer in the two families we have recruited in this study.

However, some reports failed to demonstrate a potential role of Arg72 allele in breast carcinogenesis. Any correlation was observed in Tunisian and Iranian women [17,18].

There are several potential reasons that might explain these discrepancies among reports. Differences may be due to the variation in allele frequency among different ethnic groups. According to Beckman and coworkers, the frequency of the Pro72 allele showed a north-south decline from 17% in Swedish Saamis to 63% in African Blacks (Nigerians), and there was a significant correlation between the Arg72 allele frequency and geographic latitude [24,25].

Another reason is that some studies had a very small sample size, and did not have adequate statistical power to detect potential association between Arg72 allele and breast cancer risk. On the other hand, the difference may be the inter-laboratory variation in the methods used to determine allele frequencies. Different technics were used, such as, DNA sequencing, PCR-RFLP, allele specific PCR and SSCP.

While our results highlighted the Arg72 allele as a breast cancer risk factor, we did not find any correlation between genotype distribution and clinical and histological features of breast cancer in Senegalese women. This suggested that codon72 polymorphism does not interfere with the histological pattern of breast tumors. This finding is corroborated by Papadakis et al. [26]. In contrast Tommiska et al. have reported that patients who are homozygous for the Pro72 allele have a poorer survival than patients with other genotypes [27]. Further studies in a larger sample size will confirm or infirm these results.

Conclusion

Our study showed a significant association between Arg allele of codon 72 of *p53* gene with an increased risk of developing sporadic and familial breast cancer in Senegalese women. The Arg72 allele has been implicated in the interaction between the *P53* protein with the E6 oncoprotein of Human papillomavirus (HPV) leading to cervix carcinogenesis. It would therefore be relevant to look for viral sequences of HPV in mammary cells.

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Ethical Standards

This research was approved by the Ethics Review Committee of Cheikh Anta Diop University.

Conflict of Interest

None to declare.

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