CLINICAL SCIENCE

Genomic instability at the 13q31 locus and somatic mtDNA mutation in the D-loop site correlate with tumor aggressiveness in sporadic Brazilian breast cancer cases

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OBJECTIVE: Genomic instability is a hallmark of malignant tissues. In this work, we aimed to characterize nuclear and mitochondrial instabilities by determining short tandem repeats and somatic mitochondrial mutations, respectively, in a cohort of Brazilian sporadic breast cancer cases. Furthermore, we performed an association analysis of the molecular findings and the clinical pathological data.

METHODS: We analyzed 64 matched pairs of breast cancer and adjacent non-cancerous breast samples by genotyping 13 nuclear short tandem repeat loci (namely, D2S123, TPOX, D3S1358, D3S1611, FGA, D7S820, TH01, D13S317, D13S790, D16S539, D17S796, intron 12 BRCA1 and intron 1 TP53) that were amplified with the fluorescent AmpFlSTR Identifiler Genotyping system (Applied Biosystems, USA) and by silver nitrate staining following 6% denaturing polyacrylamide gel electrophoresis. Somatic mtDNA mutations in the D-loop site were assessed with direct sequencing of the hypervariable HVI and HVII mitochondrial regions.

RESULTS: Half of the cancer tissues presented some nuclear instability. Interestingly, the D13S790 locus was the most frequently affected (36%), while the D2S123 locus presented no alterations. Forty-two percent of the cases showed somatic mitochondrial mutations, the majority at region 303-315 poly-C. We identified associations between Elston grade III, instabilities at 13q31 region ($p = 0.0264$) and mtDNA mutations ($p = 0.0041$). Furthermore, instabilities at 13q31 region were also associated with *TP53* mutations in the invasive ductal carcinoma cases ($p = 0.0207$).

CONCLUSION: Instabilities at 13q31 region and the presence of somatic mtDNA mutations in a D-loop site correlated with tumor aggressiveness.

KEYWORDS: Breast Cancer; STRs; Allelic Imbalance; LOH; Somatic mtDNA Mutation.

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INTRODUCTION

Breast cancer is the most prevalent cancer that affects women worldwide. One of the most striking characteristics of this disease is the heterogeneity of its genetic and pathological aspects (1). Genomic instability is one of the hallmarks of cancerous tissues, and it increases in advanced and more aggressive tumors (2,3). This instability may involve large chromosomal alterations, such as chromosomal deletions or duplications, and lead to allelic loss or amplification. In addition to the epigenetic mechanisms, the loss of heterozygosity (LOH), which results in allelic imbalance, is a common method of hampering tumor suppressor gene activities during carcinogenesis. *TP53* and *RB* are good examples of tumor suppressor genes that are frequently altered by allelic imbalance (3). Short tandem repeats (STRs) or microsatellites are polymorphic regions that are widely used to analyze allelic imbalance in tumors. In breast cancer, LOH has been detected at several loci in both familial and sporadic breast cancers, with frequencies ranging between 20% and 79% (4,5). Recently, Tokunaga et al. (6) studied the microsatellite instability of five randomly selected loci in Japanese primary breast cancer samples. They observed that

No potential conflict of interest was reported.

a high frequency of LOH was associated with triple-negative and high-grade HER2 breast cancers. When the same research group specifically evaluated microsatellite instability at the BRCA1 locus, they demonstrated that LOH at this region was independently associated with disease-free survival (7). In addition to nuclear genomic instabilities, researchers have also considered mitochondrial genomic alterations as indicators of cell commitment to carcinogenesis. Although their involvement is currently not well understood, somatic mitochondrial DNA (mtDNA) mutations seem to participate in cancer development in different ways (8,9). Lim et al. (10) demonstrated that mtDNA mutations in colorectal cancer might be implicated in risk factors that induce poor outcomes and tumorigenesis. Tseng et al. (11) suggested that somatic mtDNA mutations may play a critical role in breast cancer progression.

The aim of this study was to characterize nuclear instabilities and mitochondrial genomic mutations in a cohort of Brazilian sporadic breast cancer cases. We analyzed matched pairs of breast cancer and adjacent non-cancerous breast samples by genotyping 13 nuclear STR loci [namely, D2S123, TPOX, D3S1358, D3S1611, FGA, D7S820, TH01, D13S317, D13S790, D16S539, D17S796, intron 12 BRCA1 and intron 1 TP53] and by directly sequencing HVI and HVII mitochondrial regions. Furthermore, we performed an association analysis of the molecular findings and clinical pathological data from the cases.

PATIENTS AND METHODS

Tumor samples

Tissue specimens from sporadic primary breast cancer tumors and the corresponding adjacent tumor-free areas were obtained between 2005 and 2009 from the biopsies of 64 women at the Fernandes Figueira Institute, FIOCRUZ, Rio de Janeiro, Brazil. After excision, the tissues were snap-frozen in liquid nitrogen and stored at -70°C. Cancer diagnosis was confirmed by histopathology. Sixty-four percent of cases were diagnosed as invasive ductal carcinoma, and 36% were classified as invasive lobular carcinoma, mucinous, or micropapillary. DNA was extracted from the tissue samples using a salting-out method (12). The DNA was quantified using ethidium bromide staining in agarose gels and UV spectrophotometry at 260 nm. The P53 and estrogen/progesterone receptor levels, which were assessed by immunohistochemistry, and the clinical-pathological data were obtained from records of the department of pathology, IFF-FIOCRUZ. The study protocol was approved by the local ethics committee.

mtDNA sequencing

Hypervariable mitochondrial DNA regions I and II (D-loop region) were sequenced using the dideoxy chain termination method (BigDye® Terminator v3.1 Cycle Sequencing Kit) and analyzed in an automated ABI310 Sequencer (Applied Biosystems, USA). All of the sequences were aligned to the Revised Cambridge Reference Sequence, accession number NC_012920. The primer pairs designed for the PCR and direct sequencing of mtDNAs are provided in Supplementary Table 1. The mitochondrial somatic mutation data were assessed by comparing cancerous and adjacent non-cancerous breast samples.

STR typing of nuclear DNA and *TP53* mutation detection

Nuclear genomic instability was assessed by PCR analysis of 13 STR markers. The TPOX, D3S1358, FGA, D7S820, TH01, D13S317 and D16S539 loci were amplified with the fluorescent AmpFlSTR Identifiler Genotyping system according to the manufacturer's recommendations (Applied Biosystems, USA) and then analyzed using the automated ABI3100 Genetic Analyzer platform and GeneMapper Software (Applied Biosystem, USA). The D13S790 locus was amplified with an independent FAM-fluorescent system and analyzed using the ABI3100 Genetic Analyzer platform (Applied Biosystems, USA). The D2S123, D3S1611, D17S796, intron 12 BRCA1 and intron 1 TP53 loci were analyzed using silver nitrate staining following a 6% denaturing polyacrylamide gel electrophoresis. Nuclear genome instability was assessed by observing the allelic imbalances, which are usually identified as LOH. Supplementary Table 1 shows the STR loci localizations and the primer sequences. When the allelic patterns differed between the matched normal and tumor DNAs, the PCRs and electrophoresis were performed twice. Eventually, the lymphocyte DNAs of patients were also genotyped and compared to normal and tumor DNAs to confirm results. In a previous study, *TP53* mutation detection was performed for exons 4-9 (13). The association analyses were performed with Fisher's exact test with a significance level of 95% using GraphPad® software.

RESULTS

Clinical-pathological aspects of cases

To obtain all the possible noteworthy clinical-pathological data from the studied cases, the 64 patients were evaluated for age, ethnicity, histological classification, TNM, Elston grade, p53 and estrogen and progesterone receptor expression levels (Table 1 and Supplementary Tables 2 and 3). The average age of the studied patients was 53, and the ages ranged from 27 to 76 years. The ethnic classification was based on mitochondrial haplogroups. The patients were classified into three ethnic groups: African (42%), European (40%) and Asian-Amerindians (18%). Most of the cases (69%) were diagnosed as invasive ductal carcinomas (IDCs). The other histological subtypes, which represented a total of 18 cases (31%), included the following subtypes: invasive papillary carcinoma, comedocarcinoma, mucinous and medullar intraductal carcinoma. Most of the cases (75%) were classified at low or intermediate grades, although 25% were Elston grade III (high aggressiveness). Fifty percent of the cases were progesterone-positive, and 74% were estrogen-positive. In relation to the p53 tumor suppressor protein, 70% of the cases were protein-negative, and 22% were mutant (13).

Nuclear and mitochondrial genome instability

To investigate the genomic instability of our breast cancer cases, both the nuclear and mitochondrial DNAs were analyzed. Nuclear genome instabilities were detected by analyzing the forensic CODIS-recommended STR loci (i.e., D2S123, TPOX, D3S1358, FGA, D7S820, TH01, D13S317, D16S539) and the STRs that were designed for this study (i.e., D3S1611, D13S790, D17S796, intron 12 BRCA1 and intron 1 TP53). Figure 1 shows an example of LOH detection at the D13S317 locus using the fluorescent Identifiler system and a silver-stained polyacrylamide gel. Approximately half

Table 1 - Clinical-pathological aspects of the cases and an association analysis of STR instabilities and mtDNA mutations (n = 64).

Clinical-pathological		All STR instabilities			Instability at 13q31 ¤¤			Somatic mtDNA mutations §		
aspects	n	S (n = 31)	U (n = 33)	*p*-value	S (n = 37)	U (n = 27)	*p*-value	WT (n = 37)	M (n = 27)	*p*-value
Age (years)										
<55	37	19	18		21	16		22	15	
≥55	27	12	15	0.6210	16	11	1.0000	15	12	0.8017
Ethnic group										
African	27	12	15		16	11		18	9	
Non-African	37	19	18	0.6210	21	16	1.0000	19	18	0.3063
European	26	12	14		13	13		15	11	
Non-European	38	19	19	0.8035	24	14	0.3161	22	16	1.0000
AA	11	7	4		8	3		4	7	
Non-AA	53	24	29	0.3312	29	24	0.3311	33	20	0.1792
Tumor size										
≤2 cm (T1)	31	17	14		19	12		18	13	
>2 cm (T2+T3)	28	11	17	0.2994	16	12	0.7952	10	18	0.1188
Lymph node¤										
Negative	33	15	18		18	15		17	16	
Positive	26	13	13	0.7900	16	10	0.7900	11	15	0.6013
Histological subtype										
IDC	44	21	23		26	18		26	18	
Others	20	10	10	1.0000	11	9	0.7904	11	9	0.7904
Elston grade (n = 53)										
I+II	40	22	18		27	13		25	15	
III	13	4	9	0.2021	4	9	0.0264*	2	11	0.0041**
Progesterone receptor										
Positive	32	12	20		18	14		19	13	
Negative	31	19	12	0.0793	19	12	0.7994	11	20	0.0787
Estrogen receptor										
Positive	47	24	23		30	17		29	18	
Negative	16	7	9	0.7735	7	9	0.2397	1	15	0.0001**
p53										
Positive	19	7	12		9	10		7	12	
Negative	44	24	20	0.2737	28	16	0.2721	23	21	0.2866
***TP53* mutation**										
WT	50	27	23		32	18		33	17	
Mutant	14	4	10	0.1322	5	9	0.0724	4	10	0.0162*

¤¤ 13q31 region: D13S317 and D13S790 STR loci.
n - Total number of samples; S - Number of stable samples; U - Number of unstable samples; AA - Asian-Amerindian; mtDNA – Mitochondrial DNA.
WT - Wild type; M – Mutation; IDC - Invasive Ductal Carcinoma.
§Mitochondrial alteration within the D-loop region.
¤ Lymph node metastasis: Negative (N0); Positive (N1+N2+N3).
*Fisher's exact test ($p \leq 0.05$ statistically significant).
**Fisher's exact test ($p \leq 0.05$ highly statistically significant).

of the cases displayed microsatellite instability to some extent; this instability was characterized by allelic imbalances and 41% of cases exhibited alterations in three or more loci. Among the 13 analyzed STR loci, only the D2S123 locus was stable and the D7S820 locus had the lowest frequency of instability (1%). The intron 1 TP53 and D13S317 loci were each unstable in 16% of cases. Interestingly, the D13S790 locus had the highest frequency of instability among the STR loci (36%). Figure 2 displays the distribution of the number of instabilities in the STR loci. Supplementary Table

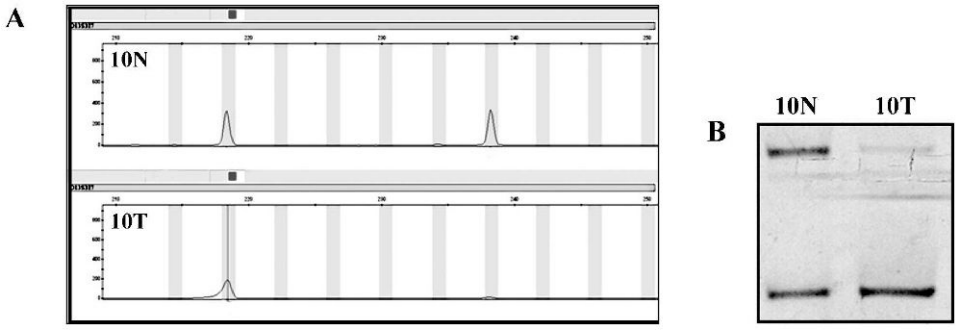

Figure 1 - Detection of LOH at the D13S317 locus. The same matched pair of samples was analyzed twice in both systems (A: Identifiler fluorescent system; and B: silver-stained polyacrylamide gel) to confirm the instability. N: normal tissue; T: tumor tissue.

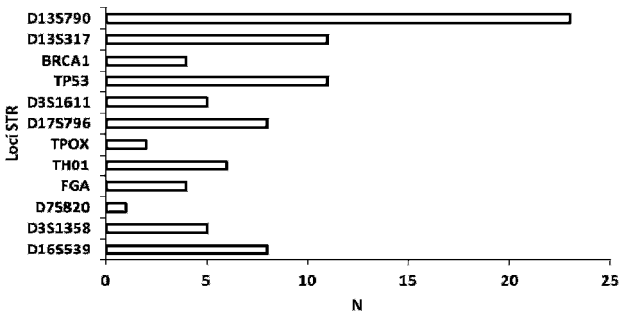

Figure 2 - Distribution of STR instabilities among the loci. The D2S123 locus presented no alterations. N: number of genetic instabilities at each STR locus.

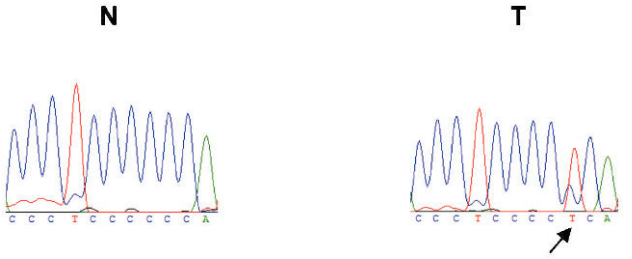

Figure 3 - Detection of the mtDNA somatic mutation (16192 CC/T) in a case of breast cancer. The arrow indicates the mutation. N: normal tissue; T: tumor tissue.

4 summarizes the data that was obtained from each of the 64 cases. Regarding the mitochondrial genome analysis, 42.18% of cases had somatic mutations, most of which were at the 303-315 poly-C region (Supplementary Table 4). Figure 3 illustrates an example of mtDNA mutation assessed by direct sequencing.

Association with clinical-pathological aspects

Following the determination of nuclear instabilities and mitochondrial genomic alterations, an association study with clinical-pathological aspects was performed. Interestingly, when the most frequent unstable genome region (13q31, assessed here through the microsatellite markers D13S317 and D13S790) was analyzed separately, it was statistically associated with Elston grade III ($p = 0.0264$) (Table 1). Furthermore, a positive association was also observed with the presence of *TP53* mutations in IDCs ($p = 0.0207$) (Table 2). A highly positive association with Elston grade III was also observed with the presence of somatic mtDNA mutations ($p = 0.0041$). Moreover, reinforcing their correlation with parameters of tumor aggressiveness, the mtDNA mutations were statistically associated with negative estrogen receptor expression ($p = 0.0001$) and *TP53* mutations ($p = 0.0162$). There was no correlation between the STR instabilities and the somatic mtDNA mutations.

DISCUSSION

Several molecular mechanisms are involved in the formation and progression of breast carcinomas, particularly sporadic breast cancers. An important feature of breast tumor development is the characteristic but highly heterogeneous genomic instability (14). Recently, the advantageous utilization of genome-scale analysis and microarray-based gene expression profiling has stressed the complexity of breast cancer progression (15,16). This study was designed and executed to provide further understanding of genomic instability in Brazilian breast cancer cases. We performed nuclear STR loci genotyping and direct sequencing of HVI and HVII mitochondrial regions of 64 matched pairs of cancerous and adjacent non-cancerous breast samples. Our main aims were to detect genomic instabilities in well-known DNA regions using selected STR loci and the mitochondrial D-loop region and to analyze their association with clinical aspects. With the results, we could expect to have a clearer understanding of local and defined genomic changes, both nuclear and mitochondrial, and their clinical consequences. Surprisingly, through the microsatellite markers D13S317 and D13S790, we found that 13q31 was the most frequent unstable genomic region. It was most apparent at the D13S790 locus, with more than 20 cases presenting LOH. When analyzed separately from the other chromosomal loci, 13q31 was shown to be statistically associated with Elston grade III in all breast tumors and with *TP53* mutations in invasive ductal carcinomas, both of which are clinical parameters of tumor aggressiveness (17,18). The 13q31 locus has been described as a chromosome region that shows different genetic alterations depending on the cancer type. Genetic gains have been observed in sarcoma (19) and colorectal cancer (20). Genetic losses have also been verified in breast cancer (21,22). Eiriksdottir et al. (23) analyzed chromosome 13q in detail in 139 sporadic breast tumors with 18 polymorphic microsatellite markers and identified 3 LOH target regions: 13q12-q13, 13q14 and 13q31-q34. In another study, correlations were

Table 2 - Association analysis of *TP53* and mtDNA mutations with STR instabilities in invasive ductal carcinoma cases (n = 44).

Clinical-pathological		All STR instabilities			Instability at 13q31¤		
aspects	n	S (n = 23)	U (n = 21)	*p*-value	S (n = 26)	U (n = 18)	*p*-value
***TP53* mutation**			14				
WT	35	21			24	11	
Mutant	9	2	7	0.0642	2	7	0.0207*
mtDNA mutations §							
WT	26	15	11		17	9	
Mutant	18	8	10	0.5406	9	9	0.3613

¤13q31region: D13S317 and D13S790 STR loci.
n – Total number of samples; S - Number of stable samples; U - Number of unstable samples; WT - Wild Type.
§Somatic mtDNA mutations within the D-loop region.
*Fisher's exact test (P≤0.05 statistically significant).

detected between the allelic loss of the D13S1694 marker (telomeric to *BRCA2*) and both larger tumor sizes and negative estrogen receptors (24). More recently, Schwarzenbach et al. (25), studying cell-free DNA in benign and malignant breast tumor cases, noted that LOH at D13S280 and D13S159, both markers located at 13q31-33, are associated with overall and disease-free survival. In this same study, all of the analyzed markers significantly correlated with lymph node status (25). Together, these results and our results suggest the existence of a putative suppressor gene or an important regulator sequence in this region. The miR17-92 cluster (13q31.3 region) is located near the 13q31 region; the cluster consists of seven microRNAs tightly grouped within an 800 bp genomic region in the third intron of the primary transcript C13orf25. This cluster is also known as oncomir-1 because its superexpression has been demonstrated in pulmonary cancer and lymphomas (26,27). However, there is some evidence of LOH in this genomic region, mainly in breast cancer, indicating that this cluster can also play a role as a tumor suppressor gene (28,29). Our results reinforce the hypothesis that instability in the 13q31 region may relate to a loss of function of microRNAs in this cluster. Because most of the allelic imbalances were associated with Elston grade III, and (more importantly) 13q31 LOH was associated with *TP53* mutations in the IDC samples, we can infer that this alteration is a delayed event in breast tumor progression. We also investigated somatic mutations in the D-loop region of the mtDNA and found that 42.18% of cases were mutated, the majority at the 303-315 poly-C region. As has been described by others (30,31), we could demonstrate an association between the presence of mtDNA mutations and breast tumor aggressiveness. Parameters such as high histological grade (Elston grade III), estrogen receptor-negative and *TP53* mutations were statistically associated. Kuo et al. (32) recently reported that the presence of somatic mutations in the D-loop indicates poor prognosis; however, they did not identify a correlation with the presence of *TP53* mutations in 30 pairs of tumor and non-tumor samples. The low number of samples and/or the different types of breast cancer cases could explain the difference. *TP53* and somatic mtDNA mutations have been considered to be good biomarkers of nuclear DNA damage (18,32); therefore, a correlation between both genetic alterations would be expected. However, we did not identify any association between nuclear instabilities and mtDNA alterations. Alazzouzi et al. (33) also observed that mitochondrial alterations were not associated with nuclear instability in breast tumors. In a study of colorectal carcinomas, instability in the 303 poly-C region of mtDNA was not associated with nuclear microsatellite instability (34). These observations suggest an independent occurrence of both phenomena. In conclusion, although the number of the Brazilian cases evaluated in this study was not high, we could highlight an important role for instabilities at the nuclear 13q31 locus and in mtDNA in breast cancer development and prognosis.

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AUTHOR CONTRIBUTIONS

Santos-Jr GC was responsible for the STR genotyping, patient data collection, statistical analysis and critical revision of the paper. Goes AC was responsible for the STR genotyping study design and execution and critical review of the manuscript. De Vitto H was responsible for mutant mtDNA design, execution and results interpretation. Moreira CC performed STR genotyping. Avad E was responsible for the patient samples and data collection. Rumjanek FD was responsible for partial financial support. De Moura Gallo CV conceived and designed the study, was responsible for research support and manuscript writing.

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APPENDIX

Supplementary Table 1 - Nuclear STR and mtDNA primer sequences.

Locus	Chromosome localization	Motif	Primer sequences		Amplicon (bp)
TPOX	2p23	AATG	ACTGGCACAGAACAGGCACTTAGG	F	224-252
			GGAGGAACTGGGAACCACAGAGGTTA	R	
D2S123	2p16	CA	AAACAGGATGCCTGCCTTTA	F	197-227
	(hMSH2)		GGACTTTCCACCTATGGGAC	R	
D3S1611	3p21	CA	CCCCAAGGCTGCACTT	F	260-268
	(hMLH1)		AGCTGAGACTACAGGCATTTG	R	
D3S1358	3p21	TCTA	ACTCGAGTCCAATCTGGTT	F	97-147
			ATGAAATCAACAGAGGCTTG	R	
FGA	4p28	TTTC	GCCCCATAGGTTTTGAACTCA	F	206-332
			TGATTTGTCTGTAATTGCCAGC	R	
D7S820	7q11	GATA	GATTCCACATTTATCCTCATTGAC	F	215-247
			ATGTTGGTCAGGCTGACTATG	R	
TH01	11p15	AATG	ATTCAAAGGGTATCTGGGCTCTGG	F	179-203
			GTGGGCTGAAAAGCTCCCGATTAT	R	
D13S790	13q31	GATA	TTGAGCCAGGATGATGTG	F	422-454
			CCTTTGGGTTGTAAACGT	R	
D13S317	13q31	TATC	ACAGAAGTCTGGGATGTGGA	F	165-197
			GCCCAAAAAGACAGACAGAA	R	
D16S539	16q24	GATA	GGGGGTCTAAGAGCTTGTAAAAAG	F	264-288
			GTTTGTGTGTGCATCTGTAAGCAT	R	
BRCA1	17q	TG	GGTCATGTGTTCCATTTGGG	F	190-270
	(intron 12 BRCA)		TTGAAGCAACTTTGCAATGAG	R	
D17S796	17p	CA	CAATGGAACCAAATGTGGTC	F	144-174
			AGTCCGATAATGCCAGGATG	R	
TP53	17p	AAAAT	GCACTGACAAAACATCCCCT	F	150-180
	(intron 1 TP53)		AGTAAGCGGAGATAGTGCCACTGT	R	
HVI	mtDNA	-	CGCACCTACGTTCAATATTACAGG	F	364
			GGTGTGTGTGTGCTGGGTAGG	R	
HVII	mtDNA	-	ATTACTGCCAGCCACCATGAA	F	445
			ACGTGTGGGCTATTTAGGCTTTA	R	

F-Forward; R-Reverse.

Supplementary Table 2 - Clinical-pathological patient data.

Case	Age (Years)	Ethnicity§	Histological classification	TNM	EG	Immunohistochemistry		
						PR	ER	P53
T2	52	African	IDC	pT1c pN0 (sn) pMx	I	+++	+++	-
T4	48	African	IDC	pT1c pN0 (sn) pMx	II	+	+	-
T5	53	African	IDC	pTis pN0 (sn) pMx	*	ND	ND	ND
T6	56	European	IDC	pT2c pN2a pMx	III	-	-	+
T8	49	African	IDC	pT1c pN0 (sn) pMx	I	+++	+++	-
T9	60	African	Invasive lobular	pT2c pN0 (sn) pMx	*	-	+++	-
T10	44	AA	IDC	pT2 pN0 pMx	III	-	-	+
T11	27	African	Intracystic papillary	pTis pN0 pMx	*	+	+++	-
T14	54	African	IDC	pT2 pN2a pMX	II	+	+++	-
T15	41	African	IDC	pT1c pN0 (sn) pMx	I	-	+++	+
T16	48	AA	IDC	pT1c pN0 (sn) pMX	I	-	+++	-
T17	46	European	IDC	pT2 pN1a pMx	II	-	-	-
T18	54	European	IDC	pT1c pN2a pMX	*	+++	+++	-
T19	50	African	Mucinous	pT1c pN0 (sn) pMX	I	-	+++	-
T21	39	AA	IDC	pT1b pN0 pMX	III	-	-	+
T23	55	African	IDC	pT2 pN1a pMx	I	+++	+++	-
T25	46	European	IDC	pT1c pN0 pMX	II	-	+++	-
T26	60	African	IDC	pT3 pN0 pMx	III	-	-	-
T27	72	African	Invasive papillary	pT1c pNx pMx	II	-	+	-
T28	46	European	IDC	pT1c pN0 (sn) pMx	III	-	+++	-
T29	70	African	Invasive papillary	pT2 pN0 (sn) pMX	I	++	+++	-
T31	36	African	Invasive micropapillary	pT2 pN1a pMx	III	-	-	-
T32	50	AA	IDC	pT1c pN0 pMx	I	-	++	-
T33	56	European	IDC	pT1c pN2a pMx	III	-	+++	-
T34	46	European	IDC	pT2 pN1a pMx	III	-	-	+
T35	49	European	IDC	pT1c pN0 (sn) pMx	II	+++	-	-
T36	53	European	IDC	pT2 pN0 (sn) pMx	II	-	-	+
T37	47	European	Mucinous	pT1c pN0 (sn) pMx	I	-	+	-
T38	61	African	IDC	pT1b pN0 (sn) pMx	I	+++	+++	-
T40	66	African	IDC	pT1c pN2 pMx	III	-	+++	+
T42	40	African	IDC	pT2 pN0 (sn) pMx	I	+++	+++	-
T43	52	AA	IDC	pTis pN0 (sn) pMx	*	-	-	-
T44	58	African	IDC	pT2 pN1a pMx	II	+++	+++	-
T46	44	European	IDC	pT2 pN3a pMx	II	-	+++	-
T47	71	European	IDC	pT2 pN0 pMx	II	+++	+++	+
T48	40	European	IDC	pT1c pN0 pMx	II	-	-	+
T50	42	African	Invasive lobular	pT1a pN1a pMx	*	++	+++	+
T52	40	European	IDC	pT2 pN1a pMx	II	++	+++	-
T53	60	European	IDC	pT1c pN1a pMx	I	-	+++	-
T55	40	European	Invasive apocrine	pT1a pN1a pMx	*	+	+	+
T56	74	AA	IDC	pT2 pN1a pMx	II	-	+++	-
T58	70	AA	Invasive lobular	pT2 pN1a pMx	*	+++	+++	-
T59	46	African	Invasive apocrine	pT2 pN1a pMx	II	-	-	+
T60	58	European	IDC	pT2 pN1a pMx	I	++	+++	-
T61	44	AA	IDC	pT2 pN1b1 pMx	II	+	+	+
T62	76	European	IDC	pT1 pN0 pMx	II	+	+	-
T63	71	African	IDC	pT1 pN1 pMx	I	+	+	-
T65	53	African	Invasive papillary	ND	II	-	+	-
T68	59	African	Invasive micropapillary	pT2 pN3 pMx	III	-	-	+
T69	72	European	Invasive lobular	pT1 pN0 pMx	*	+	+	+
T70	50	European	IDC	pT2 pN0 pMx	II	+	+	-
T71	63	European	Invasive lobular	pT1 pN1 pMx	*	+	+	+
T72	68	European	IDC	pT2 pN0 pMx	III	-	-	+
T73	63	African	Invasive papillary	pT1 pN2 pMx	III	-	-	-
T74	75	European	IDC	pT1 pN0 pMx	III	-	-	+
T75	41	European	IDC	pT2 pN1 pMx	II	+	+	-
T76	60	AA	Invasive micropapillary	pT2 pN2 pMx	II	+	+	-
T77	46	African	IDC	pT1 pN0 pMx	I	+	+	-
T78	66	European	IDC	pT1 pNx pMx	II	+	+	-
T80	28	AA	IDC	pTis pN0 pMx	*	-	+	+
T81	47	African	IDC	pT2 pN0 pMx	II	+	+	-
T82	69	European	Invasive micropapillary	pT1 pNx pMx	II	+	+	-
T83	49	African	Invasive mixed type	pT2 pNx pMx	II	+	+	-
T85	61	AA	Invasive apocrine	pT1 pN0 pMx	II	+	+	-

IDC – Invasive ductal carcinoma; TNM – Tumor-lymph node metastasis; EG – Elston grade; PR – Progesterone receptor; ER – Estrogen receptor; Protein expression: (-) negative, (+) positive - 25-50%, (++) positive - 50-75%; (+++) positive - more than 75%; ND - no data; AA - Asian-Amerindian.

*Without Elston grade classification.

§Ethnicity determined by mitochondrial haplogroup.

Supplementary Table 3 - Classification of cases according to the clinical-pathological aspects (total = 64).

Variables	Number of samples n (%)
Age (years)	
<45	14 (22)
45-55	24 (37)
55-65	13 (20)
65-75	12 (19)
>75	1 (2)
Tumor size	
T1 (≤2 cm)	31 (48)
T2 (> 2 cm)	27 (42)
T3 (> 5 cm)	1 (2)
Tis (Carcinoma *in situ*)	4 (6)
ND	1 (2)
Lymph node metastasis	
N0	33 (52)
N1	17 (26)
N2	7 (11)
N3	2 (3)
Nx	4 (6)
ND	1 (2)
Histological subtype	
IDC	44 (69)
Invasive Lobular	5 (8)
Others §	15 (23)
Elston grade*	
I	15 (28)
II	25 (47)
III	13 (25)
Progesterone receptor	
PR +	18 (28)
PR ++	4 (6)
PR +++	10 (16)
PR –	31 (48)
ND	1 (2)
Estrogen receptor	
ER +	20 (31)
ER ++	1 (2)
ER +++	26 (40)
ER –	16 (25)
ND	1 (2)
P53	
p53+	19 (30)
p53-	44 (68)
ND	1 (2)

IDC - Invasive ductal carcinoma; § Other histological subtypes - Invasive papillary, comedocarcinoma, mucinous, medullar intraductal; PR - Progesterone receptor; ER - Estrogen receptor; High levels of protein expression - +: 25-50%, ++: 50-75%, +++:>75%; -: Normal levels or low levels of protein expression; * Elston grade was applied only for the IDC subtype and other types of IDC; ND – Not detected.

Supplementary Table 4 - Unstable STR loci, mtDNA mutations and *TP53* mutation status (exons 4-9).

Case	Unstable STR loci	Mitochondrial somatic mutations	*TP53* mutation
T2	D17S796, D13S790	-	G245S
T4	D13S790	-	-
T5	D13S790	-	-
T6	-	303-315C (8-9) TC (6)	-
T8	-	16192 CC/T	-
T9	D13S790	16309 AA/G	-
T10	D3S1358, D13S317, D17S796, D3S1611, BRCA1	303-315 C (7-8) TC (6)	R248Q
T11	D13S790	-	-
T14	TH01, TP53, D3S1611, D3S1358, D17S796	303-315 C (7-8) TC (6)	-
T15	-	-	-
T16	-	303-315 C (7-8) TC (6) 16391 GG/A	-
T17	-	303-315 C (7-8) T C (6) 16261 CC/T	-
T18	-	-	R175H
T19	-	303-315 C (7-8) TC (6)	H168P
T21	FGA, D3S1358, D3S1611, D13S790	303-315 C (8-9) TC (6)	R273H
T23	-	-	-
T25	-	16192CC/T	-
T26	TP53, FGA, D16S539, D13S317	-	-
T27	-	-	-
T28	-	-	-
T29	D16S539, D17S796	146 TT/C	-
T31	FGA, D13S317, TH01, BRCA1, D13S790	-	16888delC
T32	-	-	-
T33	D13S790	-	16897-16911del
T34	D13S790	303-315 C (8-9) TC (6) 66 GG/T	Y234C
T35	D13S790, D17S796, TP53	-	-
T36	-	-	-
T37	D13S317	-	-
T38	TP53		-
T40	D13S317, FGA, TH01, D17S796, D3S1611, TP53, BRCA1, D3S1358, TPOX, D13S790	294 TT/C	I195L
T42	-	16261CC/T	-
T43	-	303-315 C (7-8) TC (6)	-
T44	-	-	-
T46	-	-	-
T47	D16S539, TP53, TPOX	-	-
T48	-	-	-
T50	TP53	-	-
T52	TP53, D13S317, D16S539, D13S790	-	-
T53	-	-	-
T55	-	294 TT/C	W146stop
T56	-	-	-
T58	-	-	-
T59	TP53, TH01, BRCA1, D3S1358, D16S539, D13S317, D13S790	303-315 C (7-8) TC (6) 338 CC/T	P278A
T60	TH01, D16S539, D13S317	-	-
T61	TP53, D13S317, D13S790	215 AA/G	-
T62	-	303-315 C (8-9) TC (6)	-
T63	-	-	-
T65	-	-	-
T68	-	-	-
T69	D13S790	303-315 C (7-8) TC (6) 338 CC/T	-
T70	D13S790	-	-
T71	D13S790	215AA/C	-
T72	D7S820, TH01, D16S539, D17S796, D13S790	303-315 C (7-8) TC (6)	R175H
T73	-	-	-

Supplementary Table 4 - Continued.

Case	Unstable STR loci	Mitochondrial somatic mutations	*TP53* mutation
T74	D13S790	303-315 C (7-8) TC (6) 16291 CC/T	-
T75	-	-	-
T76	D3S1611	303-315 C (7-8) TC	D259V
T77	-	-	-
T78	D13S790	303-315 C (7-8) TC (6) 16291CC/T	-
T80	-	-	-
T81	D13S790, D13S317	303-315 C (7-8) TC (6)	-
T82	D13S790, D16S539	-	-
T83	D13S790	-	-
T85	-	-	-