ARTICLE

The Loss of Heterozygosity of *FHIT* Gene in Sporadic Breast Cancer

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ABSTRACT

The loss of heterozygosity (LOH) is a genetic event that can change gene function. *FHIT* is a potential tumor suppressor gene. Although the precise FHIT molecular mechanism of action is not well understood, evidences suggest that Fhit protein reduced levels are involved in mammary carcinogenesis. The aim of this study was to investigate if *FHIT* LOH could influence on sporadic breast cancer (BC) biological behavior, through its association with prognostic factors for sporadic BC.

Tumor tissue and peripheral blood samples were analyzed using the microsatellite marker D3S1300. The findings were associated with clinicopathological parameters including overall survival. LOH was detected in 31.1%(52/167) of the informative BC' cases. Considering clinical and pathological characteristics we have found no significant association with *FHIT* LOH status. The mean follow-up time was 80 months. After the Cox regression analysis two parameters remained associated with BC's risk of death: TNM stage III and IV - HR = 3.74(95% CI, 1.16-12.1) P=0.027 and disease relapse HR = 3.14(CI 95% 1.26-7.80) P =0.014.

This study shows that *FHIT* LOH by itself is not a prognostic factor for sporadic BC. Further researches are required to elucidate the functional role of *FHIT* LOH concerning to BC.

1. Introduction

Breast cancer remains the most common cause of cancer-related deaths in women globally [1].

Innumerous are the efforts to find and understand the set of genetic and epigenetic changes that can influence mammary carcinogenesis and tumor biological behavior [2].

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Over the last years, advanced molecular techniques have brought to the research front opportunities to investigate new biomarkers for BC [2-3].

The *FHIT* gene is located on chromosome 3p14.2, encompassing the most common fragile site of the human genome, the FRA3B. The degree of fragility at this site may provide greater susceptibility to chromosomal rearrangements, allelic losses and breaks: alterations frequently observed in neoplastic cells [4].

Although *FHIT* function is not completely understood, this gene is considered a potential tumor suppressor. It has been involved in the pathogenesis of several tumors, including BC [5].

The loss of heterozygosis (LOH) is a genetic change commonly observed in human cancers. It occurs when a chromosomal region is lost, leading to changes in the gene function [6].

Regarding to BC the role of *FHIT* LOH is still on debate. Ahmadian *et al*. [7] inferred that *FHIT* LOH would be an early but not independent event in BC's carcinogenesis. De Oliveira *et al.* [8] have shown an association between *FHIT* LOH and ductal BC subtype. While Santos *et al*. [9] ÿÿÿÿÿÿÿÿÿÿÿÿÿÿÿÿÿÿÿ found no connections between BC's clinical features and *FHIT* LOH. Considering the potential of *FHIT* LOH as a prognostic factor for BC, only Ingvarsson *et al.* [10] reported a relationship between *FHIT* LOH and patient's survival.

In this sense, our study aimed to analyze *FHIT* LOH in a cohort of BC's patients and to investigate its possible association with clinical pathological factors and survival.

2. Materials and Methods

2.1 Study Population

The study sample was composed by paired tumor tissue and blood samples from 214 female patients diagnosed with sporadic breast cancer, from 2009 to 2014, at the Mastology Clinic of Femina Hospital, a reference women hospital in Southern Brazil.

Patients with proliferative mammary disorders, history of other types of cancer and male patients were excluded from the study.

According to the study protocol, all patients were evaluated on pre-operatory period to diagnose and stage the disease. Clinical and pathological data were filled in a standardized form. Data regarding to pathological characteristics were collected from pathology reports.

Tumor stage was established according to AJCC (2017).

2.2 Collection of Peripheral Blood and Tumor Tissue

Peripheral blood samples were collected by intravenous puncture. About 10 ml of peripheral blood in sterile tubes containing anticoagulant were collected from each patient.

Samples of the tumor tissue were obtained during the surgical procedure to treat the disease. Tumor samples were prepared for frozen sections, to check if there was sufficient amount of tumor to emit a reliable signal in the LOH analysis.

The blood and tumor tissue were packaged in sterile tubes and stored at -80ºC for later DNA extraction.

2.3 Procedures

Genomic DNA was extracted from the samples: tumor tissue and blood sample, using PureLink™Genomic DNA Mini Kit (Invitrogen™, Carlsbad, CA, USA), following manufacturer's instructions (Invitrogen Kit Handbook). In all the samples, the quality measurement of the extraction product was determined by spectrophotometry (Nano-Drop).

Polymerase chain reaction (PCR) was used to amplify the microsatellite marker D3S1300, located within FHIT gene (intron 5). The primers were constructed according to the information of reference [11] AGCTCACATTCTAGT-CAGCCT/GCCAATTCCCCAGATG and forward primer was labeled fluorescent dye 6-FAM.

The reactions were prepared to a final volume of 15 uL, containing 0.5 uL of genomic DNA, PCR buffer 1X, 1.5 mM $MgCl_2$, 200 µM of dNTPs, 750 nM of each pair of primer and 1,0 unit of platinum *Taq* DNA polymerase (Invitrogen ™, Carlsbad, CA, USA). Each microsatellite was amplified using genomic DNA (tumoral tissue and blood samples), after initial denaturation during 5 minutes at 96°C, were realized 35 cycles of the following stages: denaturation at 96°C for 30 seconds, annealing ate 55°C for 30 seconds and extension at 72°C for 1 minutes, followed by a final extension at 72°C for 20 minutes.

2.4 LOH Analysis

To verify its specificity and approximate concentration, the PCR products were analyzed by electrophoresis in agarose 1% gel stained with ethidium bromide and visualized under UV translluminator.

PCR product (1 µL) was added and homogenized with to 8.5 µL of formamide HiDi (Applied Biosystems, Foster City, CA, USA) and 0.5 of GeneScan 500 LIZ™ (Applied Biosystems, Foster City, CA, USA). Samples were denaturized for 5 minutes at 95°C and subsequently evaluated by capillary electrophoresis on ABI-PRISM 3130 (Applied Biosystems™, Foster City, CA, USA). The results were analyzed by the software GeneMapper™, version 4.0 (Applied Biosystems™, Foster City, CA, USA).

Table 1. Cox regression analysis for overall mortality.

Variables	Univariate analysis			Multivariate analysis[a-b]	
	HR (CI 95%)	*P*		HR (CI 95%)	*P*
LOH	1.40 (0.67-2.92)	<0.360			
Age, years	1.03 (0.99-1.07)	<0.067		1.01 (0.98-1.05)	<0.216
Tumour size, cm	1.22 (1.07-1.39)	<0.002		1.10 (0.96-1.26)	<0.162
Tumour grade		<0.169			
II	1.39 (0.32-5.92)				
III	2.12 (0.49-9.05)				
Histological type, lobular	1.33 (0.47-3.70)	<0.582			
TNM stage, III-IV	9.06 (3.78-21.6)	<0.001		3.74 (1.16-12.1)	<0.027
Vascular invasion	3.18 (1.41-7.15)	<0.005		1.03 (0.38-2.80)	<0.942
Molecular phenotype		<0.012			<0.647
Luminal B	2.39 (0.81-6.98)			1.58 (0.36-6.77)	
HER2	6.23 (1.68-23.1)			2.57 (0.44-14.7)	
Triple negative	3.70 (1.13-12.1)			2.21 (0.60-8.11)	
Estrogenic receptor, positive	0.41 (0.20-0.84)	<0.015		0.85 (0.17-4.23)	0.852
Ki67, >14%	1.53 (0.73-3.23)	<0.256			
Neoadjuvant chemotherapy	1.58 (0.79-3.14)	<0.193			
Disease relapse	7.88 (3.87-16.0)	<0.001		3.14 (1.26-7.80)	<0.014
Abbreviations: FHIT, fragile histidine triad; HR, hazard ratio; LOH, loss of heterozygosis. CI, confidence interval.					
[a] Included in a multivariate model variables p value was less than 0.1.					
[b] HR adjusted for age, tumour size, TNM stage, vascular invasion, molecular phenotype, estrogenic receptor and disease relapse.					

LOH was calculated as described by Van Houten *et al.* [12]. We considered LOH when we had a ratio score <0.67 or >1.35. Homozygote cases, or those with unclear results, due stutter or artifacts, were considered as non-informative (NI). Values between 0.67 and 1.35 were considered normal heterozygotes.

2.5 Statistical Analysis

Data were presented as frequency and percentage or mean ± standard deviation (SD). We performed associations between variables with the Fisher's exact test. For comparing continuous variables, a Student *t* test or an unequal variance *t* test was used. Kaplan-Meier curves for cumulative survival were compared using log rank test. Survival analysis was performed by Cox proportional hazard regression models: (i) events were defined as the time to death; (ii) censored data were used when the event did not occur at the end of the follow up period. All parameters associated with death (*P* less than 0.1) in univariate analysis were included in a multivariate model and considered statistically significant if the overall *P* value was less than 0.05. Models were adjusted all variables. The analysis supported the assumption of proportional hazard. Data were analyzed using Stata software, version 13 (Stat-

aCorp, College Station, TX, USA).

3. Results

From the total of 214 enrolled patients, 47 (22.0%) were homozygotes, non-informative (NI) cases, being excluded from the study. *FHIT* LOH was found in 52 (31.1%) of 167 heterozygotes, informative cases.

Considering clinical and pathological characteristics, *FHIT* LOH status did not present significant association with age, tumor size and grade, TNM stage, vascular invasion, estrogenic receptors status, Ki67 index, molecular phenotype and neoadjuvant chemotherapy (supplementary table).

Regarding the survival analysis, the mean follow-up time was 80 months (min. 4 - max. 96 months). During this time 33 (19.8%) patients died from BC. Since both groups (*FHIT* LOH present and absent) had homogenous clinical data, we performed a Cox regression analysis in order to investigate the role of *FHIT* LOH on patients' survival. After the multivariate analysis, only two parameters remained associated with risk of death: TNM stage III and IV – HR = 3.74 (CI 95% 1.16-12.1) P = 0.027 and disease relapse HR = 3.14 (CI 95% 1.26-7.80) P = 0.014 denoted in Table 1. Thus, as we can see in the Kaplan-Meier curve, *FHIT* LOH had no impact on patients' overall survival (Figure 1).
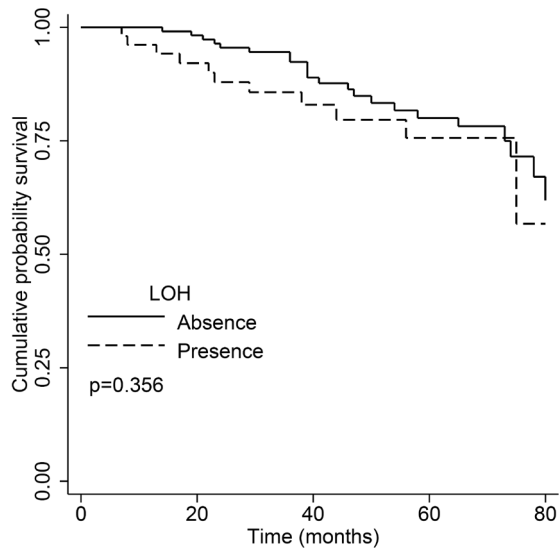
Figure 1. *FHIT* LOH status and patients' overall survival time.

4. Discussion

The precise molecular action mechanism of *FHIT* gene is still unclear, but progress is focused on understanding *FHIT* function related to tumor suppression [13-14].

Studies have pointed out that low levels of Fhit protein could be related with unfavorable parameters in BC [15-17].

LOH at the *FHIT* gene appears to disrupt its function, leading to a reduction in the gene transcripts [7,17].

In the present study, we intended to investigate if the frequency of *FHIT* LOH could influence on tumor biological behavior, through its association with prognostic and predictive factors in sporadic BC's cases.

In our study, the frequency of LOH at the *FHIT* gene detected by the intragenic marker D3S1300 was 31.1% (52 out of 167 informative cases). According to previous published data, *FHIT* LOH frequencies range from 24% to 45%, considering different BC's populations [8-9,11,18-20].

We decided to use the marker D3S1300 because it was able to detect high levels of *FHIT* LOH in prior studies [8-9,11,18,20].

Considering the clinical and pathological variables studied we found no significant association between them and *FHIT* LOH status. Similar results were also reported by Man *et al.* [19] studying low grade BC. Despite the LOH frequency of 40% detected by the marker D3S1300, no association was observed regarding age, tumor size, lymph node stage, presence of vascular invasion, menopausal status, and estrogenic receptor status. Also Santos *et al.* [9] showed no correlation of *FHIT* LOH and tumor size and grade, and axillary metastases. However, the authors highlighted the fact that from the six cases with LOH at the

intragenic marker D3S1300, four had also *BRCA1* LOH and suggested that the losses at these genes could be correlated. Subsequently the same group of researchers analyzed 3 markers for each gene LOH: *FHIT* and *BRCA1* in BC's patients and found significant clinical and prognostic association only in the cases with concomitant LOH in both genes. When the LOH was observed exclusively in one of the two genes it had none clinical impact [20]. The idea of simultaneous LOH at distinct chromosome sites is not new. It was demonstrated by Ingvarsson *et al.* [10] an association of *FHIT* LOH and the presence of LOH at other 12 chromosome regions. We should also keep in mind that *FHIT* is located in an active fragile site, the FRA3B. Because of its genomic instability, genetic alterations in other genomic sites could influence on it, predisposing to losses and breaks [14,16,21]. It is also in consonance with the concept of multistep carcinogenesis, in which cancer results from an accumulation of mutations [22].

Few studies analyzed *FHIT* locus changes and patient survival. Some of these use distinctive methodologies and included various tumors types, such as cervical, lung, colon, and gastric cancers [23-25]. When it comes to the LOH at *FHIT* gene and BC´s patients' survival, studies are restricted to two.

Silva Soares *et al.* [20] have shown reduced survival time after 48 months of follow-up of 72 BC's patients who have concurrent *BRCA1* and *FHIT* LOH (P=0.04). Patients with *FHIT* LOH alone had no differences regarding to survival time. The other study including patients' survival analysis enrolled 239 women with BC. After a mean follow-up time of 5 years the authors reported a relative risk of dying for patients with *FHIT* LOH of 1.6 (95% CI: 1.0-2.6) P=0.45. [10] However, despite the effect of *FHIT* LOH on patients' survival, the study sample was composed by women with sporadic and familial BC. From the total of 76 cases with *FHIT* LOH, 30 cases were carriers of BRCA2 999de15 germ line mutation. And besides, it was observed a significant correlation between *FHIT* LOH and ER and PR negativity, which are also parameters associated to familial BC. Our study included 167 informative cases. We intend to analyze if the LOH at the *FHIT* gene alone could impact on clinical pathologic characteristics and patients survival, considering a large sample of exclusively sporadic BC. After multivariate analysis our results evidenced nothing but two well-established independent prognostic factors for BC: TNM stage and disease relapse. The *FHIT* LOH had no significant association with patients' prognoses. This could be due to the fact that we used only one microsatellite marker. Nevertheless we have chosen an accurate marker according to previous studies and the frequency of 31% of *FHIT*

LOH we detected is within the range published before. According to our data, LOH at the *FHIT* gene is not related to clinical parameters for BC. So we can infer that other mechanisms could explain the association between low levels of Fhit protein and adverse biological behavior demonstrated in earlier researches [15-17,26].

Yang *et al.* [27] have tested the two-hit theory for *FHIT* complete inactivation and found a high index of concordance among LOH (first-hit), promoter hypermethylation (second-hit) and absent Fhit protein expression in BC samples. The authors reported that the *FHIT* hypermethylation was an event much more frequent than LOH in BC. These findings empower the model of biallelic inactivation requirement for the whole silencing of a tumor suppressor gene [28]. And, at the same time, it raises the hypothesis that demethylating-therapy could play a role for such tumors.

Other possibilities cannot be ruled out, as splicing abnormalities [13,29] and coexistent allelic losses at chromosome 3p. [30] Also *FHIT* point mutations, though rarely can occur, and influence on the gene transcripts [7].

Knowledge about the *FHIT* function and role in breast tumors are still not fully explained. Considering that alterations in Fhit protein may have implications in mammary carcinogenesis, further studies are needed to explore the mechanisms whereby these changes occur.

5. Conclusions

Our results show that *FHIT* LOH by itself is not a prognostic factor for BC. However efforts must remain to elucidate the functional significance of LOH at *FHIT* gene and its connection to BC.

The variable frequencies of LOH found in other studies can be explained by some factors, such as different methodologies in which other studies evaluate LOH in samples in paraffin blocks. In different types of samplings, such as sporadic breast cancer, hereditary breast cancer, bilateral breast cancer, benign breast disorders compared to malignant. Another relevant factor is that some studies have evaluated other microsatellite markers that may have been a limitation of our study. We also hypothesized that different results could be found if the study population were hereditary breast cancer. As a future prospect we intend to evaluate *FHIT* gene methylation and protein expression.

Ethics Approval

All the patients were informed about the study and signed the Consent Form, approved with the project, by the Committee of Ethics on Research (Protocol number: 10-641).

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018. 68 (2018) 394-424.
DOI: 10.3322/caac.21492.

[2] Pedraza V, Gomez-Capilla JA, Escaramis G, et al. Gene expression signatures in breast cancer distinguish phenotype characteristics, histologic subtypes, and tumor invasiveness, Cancer. 116 (2010) 486-496.
DOI: 10.1002/cncr.24805.

[3] Weigelt B, Baehner FL, Reis-Filho JS. The contribution of gene expression profiling to breast cancer classification, prognostication and prediction: A retrospective of the last decade, J Pathol. 220 (2010) 263-280.
DOI: 10.1002/path.2648.

[4] Glover TW, Stein CK. Chromosome breakage and recombination at fragile sites, Am J Hum Genet. 43 (1988) 265-273. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1715373/

[5] Pekarsky Y, Palamarchuk A, Huebner K, Croce CM. Fhit as tumor suppressor: Mechanisms and therapeutic opportunities, Cancer Biol Ther. 1 (2002) 232-236.
DOI: 10.4161/cbt.73.

[6] Lasko D, Cavenee W, Nordenskjold M. Loss of constitutional heterozygosity in human cancer, Annu Rev Genet. 25 (1991) 281-314.
DOI: 10.1146/annurev.ge.25.120191.001433.

[7] Ahmadian M, Wistuba II, Fong KM, et al. Analysis of the fhit gene and fra3b region in sporadic breast cancer, preneoplastic lesions, and familial breast cancer probands, Cancer Res. 57 (1997) 3664-3668. https://cancerres.aacrjournals.org/content/57/17/3664.long.

[8] de Oliveira MM, de Oliveira SF, Lima RS, et al. Differential loss of heterozygosity profile on chromosome 3p in ductal and lobular breast carcinomas, Hum Pathol. 43 (2012) 1661-1667.
DOI: 10.1016/j.humpath.2011.12.008.

[9] Santos SC, Cavalli LR, Cavalli IJ, Lima RS, Haddad BR, Ribeiro EM. Loss of heterozygosity of the brca1 and fhit genes in patients with sporadic breast cancer

from southern brazil, J Clin Pathol. 57 (2004) 374-377.
DOI: 10.1136/jcp.2003.013490.

[10] Ingvarsson S, Sigbjornsdottir BI, Huiping C, Jonasson JG, Agnarsson BA. Alterations of the fhit gene in breast cancer: Association with tumor progression and patient survival, Cancer Detect Prev. 25 (2001) 292-298. https://www.researchgate.net/publication/11915856.

[11] Rabelo RA, Antunes LM, Etchebehere RM, et al. Loss of heterozygosity in the fragile histidine triad (fhit) locus and expression analysis of fhit protein in patients with breast disorders, Clin Exp Obstet Gynecol. 40 (2013) 89-94. https://pubmed.ncbi.nlm.nih.gov/23724516/.

[12] Van Houten VM, Tabor MP, van den Brekel MW, et al. Molecular assays for the diagnosis of minimal residual head-and-neck cancer: Methods, reliability, pitfalls, and solutions, Clin Cancer Res. 6 (2000) 3803-3816. https://clincancerres.aacrjournals.org/content/6/10/3803.long.

[13] Negrini M, Monaco C, Vorechovsky I, et al. The fhit gene at 3p14.2 is abnormal in breast carcinomas, Cancer Res. 56 (1996) 3173-3179. https://cancerres.aacrjournals.org/content/56/14/3173.long.

[14] Croce CM, Sozzi G, Huebner K. Role of fhit in human cancer, J Clin Oncol. 17 (1999) 1618-1624.
DOI: 10.1200/JCO.1999.17.5.1618.

[15] Yang Q, Yoshimura G, Suzuma T, et al. Clinico-pathological significance of fragile histidine triad transcription protein expression in breast carcinoma, Clin Cancer Res. 7 (2001) 3869-3873. https://clincancerres.aacrjournals.org/content/7/12/3869.long.

[16] Campiglio M, Pekarsky Y, Menard S, Tagliabue E, Pilotti S, Croce CM. Fhit loss of function in human primary breast cancer correlates with advanced stage of the disease, Cancer Res. 59 (1999) 3866-3869. https://cancerres.aacrjournals.org/content/59/16/3866.long.

[17] Ingvarsson S, Agnarsson BA, Sigbjornsdottir BI, et al. Reduced fhit expression in sporadic and brca2-linked breast carcinomas, Cancer Res. 59 (1999) 2682-2689. https://cancerres.aacrjournals.org/content/59/11/2682.long.

[18] Kollias J, Man S, Marafie M, et al. Loss of heterozygosity in bilateral breast cancer, Breast Cancer Res Treat. 64 (2000) 241-251.
DOI: 10.1023/a:1026575619155.

[19] Man S, Ellis IO, Sibbering M, Blamey RW, Brook JD. High levels of allele loss at the fhit and atm genes in non-comedo ductal carcinoma in situ and grade i tubular invasive breast cancers, Cancer Res. 56 (1996) 5484-5489. https://cancerres.aacrjournals.org/content/56/23/5484.long.

[20] Silva Soares EW, de Lima Santos SC, Bueno AG, et al. Concomitant loss of heterozygosity at the brca1 and fhit genes as a prognostic factor in sporadic breast cancer, Cancer Genet Cytogenet. 199 (2010) 24-30.
DOI: 10.1016/j.cancergencyto.2010.01.019.

[21] Huebner K, Croce CM. Fra3b and other common fragile sites: The weakest links, Nat Rev Cancer. 1 (2001) 214-221.
DOI: 10.1038/35106058.

[22] Barrett JC. Mechanisms of multistep carcinogenesis and carcinogen risk assessment, Environ Health Perspect. 100 (1993) 9-20.
DOI: 10.1289/ehp.931009.

[23] Burke L, Khan MA, Freedman AN, et al. Allelic deletion analysis of the fhit gene predicts poor survival in non-small cell lung cancer, Cancer Res. 58 (1998) 2533-2536. https://cancerres.aacrjournals.org/content/58/12/2533.long.

[24] Petursdottir TE, Hafsteinsdottir SH, Jonasson JG, et al. Loss of heterozygosity at the fhit gene in different solid human tumours and its association with survival in colorectal cancer patients, Anticancer Res. 22 (2002) 3205-3212. https://pubmed.ncbi.nlm.nih.gov/12530066/.

[25] Capuzzi D, Santoro E, Hauck WW, et al. Fhit expression in gastric adenocarcinoma: Correlation with disease stage and survival, Cancer. 88 (2000) 24-34. https://pubmed.ncbi.nlm.nih.gov/10618602/.

[26] Gatalica Z, Lele SM, Rampy BA, Norris BA. The expression of fhit protein is related inversely to disease progression in patients with breast carcinoma, Cancer. 88 (2000) 1378-1383. https://pubmed.ncbi.nlm.nih.gov/10717620/.

[27] Yang Q, Nakamura M, Nakamura Y, et al. Two-hit inactivation of fhit by loss of heterozygosity and hypermethylation in breast cancer, Clin Cancer Res. 8 (2002) 2890-2893. https://clincancerres.aacrjournals.org/content/8/9/2890.long.

[28] Knudson AG, Jr. Mutation and cancer: Statistical study of retinoblastoma, Proc Natl Acad Sci U S A. 68 (1971) 820-823.
DOI: 10.1073/pnas.68.4.820.

[29] Hayashi S, Tanimoto K, Hajiro-Nakanishi K, et al. Abnormal fhit transcripts in human breast carcinomas: A clinicopathological and epidemiological analysis of 61 japanese cases, Cancer Res. 57 (1997) 1981-1985. https://cancerres.aacrjournals.org/con-

tent/57/10/1981.long.

[30] Martinez A, Walker RA, Shaw JA, Dearing SJ, Maher ER, Latif F. Chromosome 3p allele loss in early invasive breast cancer: Detailed mapping and association with clinicopathological features, Mol Pathol. 54 (2001) 300-306.

DOI: 10.1136/mp.54.5.300.

Supplementary Table

Clinical and pathological characteristics and LOH status of the sample.				
Variables	Total (N=167)	LOH		*P*
		Absence (n=115)	Presence (n=52)	
Age, years	55.95±11.96	56.24±11.42	55.29±13.170	<0.586
Tumour size, cm	2.93±2.24	2.69±1.83	3.48±2.910	<0.220
Tumour grade				<0.808
I	18 (10.8)	13 (11.3)	05 (9.6)0	
II	83 (49.7)	55 (47.8)	28 (53.8)	
III	66 (39.5)	47 (40.9)	19 (36.5)	
TNM stage				<0.053
I-II	109 (65.3)0	81 (70.4)	028 (53.8)	
III-IV	58 (34.7)	34 (29.6)	024 (46.2)	
Vascular invasion				<0.228
No	77 (51.3)	55 (55.0)	22 (44.0)	
Yes	73 (48.7)	45 (45.0)	28 (56.0)	
Molecular phenotype				<0.988
Luminal A	51 (31.1)	31 (27.4)	20 (39.2)	
Luminal B	73 (44.5)	57 (50.4)	16 (31.4)	
HER2	10 (6.1)0	06 (5.3)0	04 (7.8)0	
Triple negative	30 (18.3)	19 (16.8)	11 (21.6)	
Estrogenic receptor				0.333
Negative	40 (24.0)	25 (21.7)	15 (28.8)	<
Positive	127 (76.0)0	90 (78.3)	37 (71.2)	
Ki67				<0.220
14%<	68 (43.9)	43 (40.2)	25 (52.1)	
>14%	87 (56.1)	64 (59.8)	23 (47.9)	
Neoadjuvant chemotherapy				<0.715
No	117 (70.1)0	82 (71.3)	35 (67.3)	
Yes	50 (29.9)	33 (28.7)	17 (32.7)	
Note: Data were presented as No. (%) or mean ± SD.				