Copy number alternations of the 17q23-rs6504950 locus are associated with advanced breast cancers in Taiwanese women

Chien-Yu Lin, Shu-Fen Yang, Yu-Ling Ho, Cheng-Mao Ho

ABSTRACT

Objective: Breast cancer is one of the most common malignancies and a leading cause of cancer-related death in women worldwide. Both hormone-related factors and genetic aberrations could cause breast cancer. We investigated copy number alternations (CNAs) on four breast cancer-susceptible loci, namely 2q35-rs13387042, 3p24-rs4973768, 17q23-rs6504950, and fibroblast growth factor receptor 2 (FGFR2)-rs2981578, in Taiwanese women. Patients and Methods: Breast cancer tissues and blood samples from 66 patients and their clinical data were collected from a human biobank. The copy numbers of the germline samples (from blood) and cancer tissues from each patient on the susceptible loci – 2q35, 3p24, 17q23, and FGFR2 – were obtained using TaqMan probes in the Applied Biosystems Inc., (ABI) StepOnePlus Real-Time Polymerase Chain Reaction instrument and CopyCaller® Software v1.0 (ABI, CA, USA). Results: The mean copy numbers output by CopyCaller® Software v1.0 of the cancer tissues on these susceptible loci (2q35, 3p24, 17q23, and FGFR2) from the 66 patients were higher than those of the blood samples (2.0 vs. 1.9); however, significantly higher copy numbers for cancer tissues compared with germline samples were discovered only on 2q35-rs13387042 (P = 0.035). In addition, patients with advanced breast cancers had relatively many CNAs between their cancer tissues and germline samples on 17q23-rs6504950 (P = 0.008). Multivariate analysis revealed that the risk factor for patients with advanced breast cancers was CNAs between cancer tissues and germline samples on 17q23-rs6504950 (odds ratio = 13.337, 95% confidence interval: 1.525–122.468). Conclusions: CNAs on 17q23-rs6504950 between cancer tissues and germline samples could affect cancer progression in Taiwanese women with breast cancer. Further investigations regarding the role of CNAs on 17q23-rs6504950 in cancer progression are necessary to elucidate the pathogenesis of breast cancer.

KEYWORDS: 17q23, Breast cancer, Copy number alternations, Taiwan

INTRODUCTION

According to the GLOBOCAN database of the International Agency for Research on Cancer, part of the World Health Organization (http://globocan.iarc.fr/), breast cancer, a hormone-dependent disease, was one of the most common malignancies and causes of cancer-related death in women worldwide in 2012 (based on global estimated cancer incidence, mortality, and prevalence). According to the Taiwan Health and Welfare Report 2017, the most common cancer among women in Taiwan is breast cancer, with an age-standardized incidence rate of 0.707%. In addition, breast cancer is the fourth leading cause of death among female malignancies, with age-standardized mortality rate of 0.128% [1]. Although East Asia, including Taiwan, has a relatively low rate of breast cancer compared with North America and Western Europe, breast cancer incidence is increasing in all regions of the world because of the westernization of developing regions [2]. An age–period–cohort analysis revealed that the incidence of breast cancer in Taiwan was increasing, especially among young patients; the median age of patients with breast cancer in Taiwan (45–49 years) is younger than that in Western countries (70–74 years) [3].

Breast cancer has multiple risk factors; among these factors, hormone-related factors and genetic relationships

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are notable [4-6]. Hormone-related factors include age, sex, menarche, menopause, hormone therapy, breastfeeding, and contraceptive use [4]. Various gene aberrations, including germline or somatic changes, have been verified. Somatic mutations of TP53, PIK3CA, and GATA3 were identified by various molecular platforms [6]. Several hereditary cancer syndromes were found to be associated with breast cancer, including hereditary breast and ovarian syndrome (BRCA1 and BRCA2), familial diffuse gastric cancer syndrome (CDH1), Cowden syndrome (PTEN), Peutz-Jeghers syndrome (STK11 or LKB1), and Li-Fraumeni syndrome (TP53) [5,7]. In addition, linkages between single-nucleotide polymorphisms (SNPs) and breast cancer were elucidated after genome-wide association studies (GWASs). Several loci susceptible to breast cancer have been detected, including 2q35-rs13387042, 3p24-rs4973768, 17q23-rs6504950, and fibroblast growth factor receptor 2 (FGFR2)-rs2981578 [8-10]. In addition to genetic mutations and SNP-susceptible loci, copy number variation may contribute to clinical phenotypes and might be associated with disease risk [11]. Except for BRCA1 and BRCA2 and several SNP-susceptible loci, genetic aberrations in breast cancers are rarely evaluated in Taiwan [12,13]. In the present study, we investigated copy number alternations (CNAs) on the aforementioned four breast cancer-susceptible loci (2q35-rs13387042, 3p24-rs4973768, 17q23-rs6504950, and FGFR2-rs2981578) in Taiwanese women with breast cancer as well as associations between various genetic aberrations and clinical features.

**PATIENTS AND METHODS**

**Study population and clinical data**

Breast cancer samples from 66 patients and their clinical data were collected from the human biobank of China Medical University Hospital. This research project was reviewed and approved by the Institutional Review Board of China Medical University Hospital (DMR99-IRB-108). The clinical characteristics of the female breast cancer patients are presented in Table 1 alongside the patients’ clinical indices, including age (mean and range), tumor, node and metastasis (TNM) stage (early stage and advance stage), estrogen receptor (ER) positivity, progesterone receptor (PR)-positivity, and human epidermal growth factor receptor 2 (HER2) positivity.

**DNA extraction**

DNA was extracted from cancer tissue and germline samples (blood) using a commercial kit (QIAamp DNA Micro Kit and QIAamp DNA Blood Mini Kit, QIAGEN GmbH, D40724 Hilden). After extraction, the DNA was quantified using a conventional spectrophotometric method with absorbance measurements at 260 nm (A260). The DNA concentration was between 50 and 300 ng/µL.

**Copy number alternations on 2q35-rs13387042, 3p24-rs4973768, 17q23-rs6504950, and fibroblast growth factor receptor 2-rs2981578**

The real-time polymerase chain reaction (PCR) was performed using TaqMan probes in the Applied Biosystems Inc., (ABI) StepOnePlus Real-Time PCR instrument (ABI, CA, USA). These probes are commercially available from TaqMan (ABI). Commercially available FAM dye-labeled probes were designed to amplify the 2q35-rs13387042, 3p24-rs4973768, 17q23-rs6504950, and FGFR2-rs2981578. A VIC dye-labeled ribonuclease P RNA component H1 (RPPH1) was used as the endogenous control because RPPH1 has exactly two copies per diploid human genome; this genome is located on chromosome 14q11.2. The TaqMan copy number assay contained a 0.5-µL probe (20, FAM labeled), 0.5-µL probe mix (+20, VIC labeled), 5-µL TaqMan Universal PCR Master Mix (+2), 1 µL of genomic DNA (10 ng/µL), and 3 µL of water. The amplification protocol used for the reaction was 95°C for 10 min, followed by 95°C for 15 s and then 60°C for 1 min for 40 cycles. The sequences of primers and probes of the four loci are shown in Table 2.

A manual cycle threshold of 0.2 and an automatic baseline were used to detect the template quantity of the target genes and RPPH1 gene in sequence detection system software (Applied Biosystems CopyCaller Software v2.0). For each sample, four probes (2q35-rs13387042, 3p24-rs4973768, 17q23-rs6504950, and FGFR2-rs2981578) were performed alongside an internal control. The target probes and internal controls were assigned a manual cycle threshold of 0.2 and an automatic baseline.

**Table 1: Characteristics of 66 patients with breast cancer**

| Characteristics | n (%) |
|-----------------|-------|
| Age (years)     |       |
| Mean±SD         | 53±12 |
| Range           | 33-85 |
| TNM stage a     |       |
| Early stage (I/II) | 42 (63.3) |
| Advanced stage (III/IV) | 24 (36.4) |
| ER-positive     | 37 (56.1) |
| PR-positive     | 53 (80.3) |
| HER2-Positive   | 34 (51.5) |

aTNM stages are based on the American Joint Committee on Cancer’s Cancer Staging Manual, 6th Edition (2002). ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2, TNM: Tumor, node and metastasis, SD: Standard deviation

**Table 2: Primers-probe sequences of genes analyzed for copy number assay**

| Gene name         | Primer/probe sequence (5’‑3’) |
|-------------------|------------------------------|
| 2q35-rs13387042   | Forward primer               |
|                   | CCAGAACAAAGAAGGCAAATGGA     |
|                   | Reverse primer               |
|                   | GTGGTGAAAGGAGATTGAAAGAAGA   |
|                   | Probe                        |
|                   | FAM'-CTACAGAAACCAAGGATTCC   |
| 3p24-rs4973768    | Forward primer               |
|                   | GTGAGAGATTATATACCTTTAAAACAGCGT |
|                   | Reverse primer               |
|                   | GACTCTTGACTGCAAATAGTGCTGACT |
|                   | Probe                        |
|                   | FAM'-AACATGAGTTACCTTTTGTCCTAAATG |
| 17q23-rs6504950   | Forward primer               |
|                   | ACTCCACCCACACGCAAGATATT    |
|                   | Reverse primer               |
|                   | GGGAAATGGGATATCAGCAATGG    |
|                   | Probe                        |
|                   | FAM'-TATCCCTGGCTTTGATAGACAAAC |
| FGFR2-rs2981578   | Forward primer               |
|                   | GCAAGGAGAGGAAAGGTAACTGTGAF  |
|                   | Reverse primer               |
|                   | CTGAGCCAGAGGACTGAACC        |
|                   | Probe                        |
|                   | FAM'-ACGTGGAATGTCCTGAATTA   |
control were loaded at the same well, and each reaction was performed in triplicate. CopyCaller software (ABI, version 1.0) was used to calculate the integer copy number of each probe based on the real-time PCR data.

If the copy number difference output by CopyCaller software (ABI, version 1.0) between each patient germline sample and cancer tissue was ≥0.50–1.49, we considered that there were one CNA between germline sample and cancer tissue in the clinical characteristics analysis.

Statistical analyses

Pearson’s Chi-square test or Fisher’s exact test (when the expected number in any cell was lower than five) was used to determine associations between different genetic parameters and clinical cancer stages. For continuous variables such as copy numbers, t-tests were conducted. All statistics were calculated using the Statistical Package for the Social Sciences for Windows (version 17.0; Chicago, IL, USA). \( P \leq 0.05 \) was considered statistically significant. All significance tests were two tailed.

RESULTS

The copy numbers of cancer tissues and germline samples from the 66 patients on four breast cancer-susceptible loci (2q35-rs13387042, 3p24-rs4973768, 17q23-rs6504950, and FGFR2-rs2981578) are shown in Figure 1. The copy numbers output by CopyCaller software (ABI, version 1.0) on these four loci of the cancer tissues seemed more aberrant compared to those of the germline samples. A statistical copy number change was observed on only 2q35-rs13387042 (\( P = 0.035 \)).

Table 3 shows the various clinical characteristics of the breast cancer patients at early (Stages I and II) or advanced (Stages III and IV) stages. No significant differences were observed between the early- and advanced-stage patients with respect to menopause (\( P = 0.611 \)), ER presence (\( P = 1.000 \)), PR presence (\( P = 0.345 \)), HER2 receptor presence (\( P = 0.451 \)), or CNAs (between cancer tissues and blood samples in each patient) on the FGFR2-rs2981578 (\( P = 0.700 \)), 2q35-rs13387042 (\( P = 1.000 \)), or 3p24-rs4973768 (\( P = 0.374 \)) locus. However, more patients with advanced cancers had CNAs on the 17q23-rs6504950 locus (\( P = 0.008 \)). Multivariate analysis with adjustment for age revealed only one significant risk factor in patients with advanced breast cancers, namely CNAs on the 17q23-rs6504950 locus (odds ratio [OR] = 13.337, 95% confidence interval [CI]: 1.525–122.468) [Table 4].

DISCUSSION

Several GWASs in Western countries have provided information regarding breast cancer-susceptible loci, including 2q35, 3p24, 17q23, and FGFR2 [8,9,14-19]. However, associations between breast cancer and CNAs on these loci have not been investigated among the Taiwanese population. In the present study, we intended to elucidate the roles of CNAs on these four breast cancer-related loci in Taiwan. Comparison of copy numbers of these four loci between cancer tissues and germline samples revealed significant differences only on the 2q35-rs13387042 locus, which was near genes LOC101928278 and LOC105373874 on chromosome 2, with a global minor allele frequency (MAF; A allele) of 0.4742. In our previous study, 2q35-rs13387042 of allele A conferred a higher breast cancer risk than did allele G (OR = 2.95, 95% CI: 1.29–6.71, \( P = 0.008 \)) [13]. In a study of Caucasian women from Europe, a polymorphism on 2q35-13387042 was associated with ER-positive breast cancer (OR = 1.14, 95% CI: 1.10–1.17) [9].

| Parameter                | Early stage (n=42) | Advanced stage (n=24) | \( P \)  |
|--------------------------|--------------------|-----------------------|---------|
| Age (menopause age\( ^a \)) |                    |                       |         |
| ≤49 year-old (n=31)  | 21 (50.0)          | 10 (41.7)             | 0.611   |
| >49 year-old (n=35)  | 21 (50.0)          | 14 (58.3)             |         |
| Estrogen receptor      |                    |                       |         |
| Negative (n=29)       | 18 (42.9)          | 11 (45.8)             | 1.000   |
| Positive (n=37)       | 24 (57.1)          | 13 (54.2)             |         |
| Progesterone receptor  |                    |                       |         |
| Negative (n=13)       | 10 (23.8)          | 3 (12.5)              | 0.345   |
| Positive (n=53)       | 32 (76.2)          | 21 (87.5)             |         |
| HER2 receptor          |                    |                       |         |
| Positive (n=32)       | 22 (52.4)          | 10 (41.7)             | 0.451   |
| Negative (n=34)       | 20 (47.6)          | 14 (58.3)             |         |
| CNAs on FGFR2-rs2981578 |                  |                       |         |
| No (n=58)             | 36 (85.7)          | 22 (91.7)             | 0.700   |
| Yes (n=8)             | 6 (14.3)           | 2 (8.3)               |         |
| CNAs on 2q35-rs13387042 |                |                       |         |
| No (n=42)             | 27 (64.3)          | 15 (62.5)             | 1.000   |
| Yes (n=24)            | 15 (35.7)          | 9 (37.5)              |         |
| CNAs on 3p24-rs4973768 |                |                       |         |
| No (n=51)             | 34 (81.0)          | 17 (70.8)             | 0.374   |
| Yes (n=15)            | 8 (19.0)           | 7 (29.2)              |         |
| CNAs on 17q23-rs6504950 |                |                       |         |
| No (n=59)             | 41 (97.6)          | 18 (75.0)             | 0.008*  |
| Yes (n=7)             | 1 (2.4)            | 6 (25.0)              |         |

\( ^a \)The menopausal age cutoff point was based on data from the Health Promotion Administration, Ministry of Health and Welfare, Taiwan. HER2: Human epidermal growth factor receptor 2, CNA: Copy number alternation

Figure 1: Copy numbers (output from CopyCaller® Software v1.0) of germline samples (blood samples) and tumor samples (cancer tissues) from the 66 breast cancer patients on four susceptible loci: fibroblast growth factor receptor 2-rs2981578, 2q35-rs13387042, 3p24-rs4973768, and 17q23-rs6504950 (*\( P \leq 0.05 \)). Germline sample (blood sample). Tumor samples (cancer tissues)
We observed that patients with advanced breast cancers had more CNAs (based on the comparison between cancer tissues and germline samples) on the 17q23-rs6504950 locus ($P = 0.008$) than did those with early-stage cancers. In addition, multivariate logistic regression analysis with age adjustment revealed only one risk factor of advanced breast cancer, namely CNAs on the 17q23-rs6504950 locus (OR = 13.337, 95% CI: 1.525–122.468). The 17q23-rs6504950 locus was in the STXBP4 gene (also named COX11) on chromosome 17, with a global MAF (A allele) of 0.2304. A previous GWAS discovered strong evidence for additional susceptible loci, namely 3p24-rs4973768 (OR = 1.11, 95% CI: 1.08–1.13, $P = 4.1 \times 10^{-25}$) and 17q23-rs6504950 (OR = 0.95, 95% CI: 0.92–0.97, $P = 1.4 \times 10^{-9}$) [8].

The potential causative genes were SLC4A7 and NEK10 on 3p and COX11 on 17q. Notably, a cohort study that evaluated eight GWAS-identified SNPs (rs2981582, rs1219648, rs3803662, rs12443621, rs8051542, rs999737, rs6504950, and rs49737680) among 739 Caucasian women with early-stage breast cancers suggested that previously identified breast cancer-susceptible loci, namely rs12443621 and rs6504950, might influence the prognoses, comorbid conditions, and overall survival of patients with breast cancer [20]. There were also previous studies disclose the poor prognostic effect of CNAs on the 17q23 loci on breast cancer [21,22]. However, a meta-analysis that evaluated the STXBP4/COX11 rs6504950 polymorphism (AA/AG genotypes) in 17,960 cases and 22,713 controls yielded a contradictory result with significantly lower risk of breast carcinogenesis (OR = 0.87–0.92) [23].

**CONCLUSION**

In the present study, we observed that copy numbers on the 2q35-rs13387042 locus of breast cancer tissues were significantly higher than those of blood samples in our 66 patients with breast cancer ($P = 0.035$). CNAs on the 17q23-rs6504950 locus might be a risk factor (OR = 13.337, 95% CI: 1.525–122.468) for patients with advanced cancers in Taiwan. However, there were several limitations in our study: TaqMan Probe assay compared to MLPA (Multiplex Ligation-dependent Probe Amplification) was not an exact method for CNAs analysis [24], small sample size and retrospective study. Further investigations on the role of CNAs on 17q23-rs6504950 in breast cancer progression are necessary to elucidate the pathogenesis of breast cancer.

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**Conflicts of interest**

There are no conflicts of interest.

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