

# Gain in 1q is a common abnormality in phyllodes tumours of the breast

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**Abstract.** We studied DNA copy number changes by CGH and allelic imbalance (AI) on 3p by LOH analysis on 22 phyllodes tumours (PT) of the breast in order to gain insight into the genetic basis of tumour progression in PT. Copy number changes were observed in 14 cases (63%). Gain in 1q with 1q21–23 as the minimal overlapping area was seen in 12 cases (55%). The gain was observed both in benign and malignant tumours. Our study did not reveal any DNA copy number changes or allelic loss on 3p. The results suggest that DNA copy number changes are not associated with the histological grade or clinical behaviour of PT and the chromosomal changes on 3p appear to be rare.

Colour figure can be viewed on <http://www.esacp.org/acp/2003/25-2/jee.htm>.

Keywords: Phyllodes tumour, comparative genomic hybridization, allelic loss on 3p, chromosome 1q21–q23

## 1. Introduction

Phyllodes tumours (PT) are rare neoplasms of the breast that account for less than 1% of all breast tumours [1]. The histologic classification between benign and malignant PT is difficult and arbitrary [2]. Most of these tumours can display locally destructive growth and even metastasise [3]. However, the clinical course of PT is unpredictable and does not correlate with the histological parameters. Recent studies have suggested that some markers, for example, ki-67, p53 and CD34, may be clinically useful in classification and diagnosis of PT [4–6]. Cytogenetic investigations have shown various and complex alterations in a small number of PT [7–10].

So far only one comparative genomic hybridization (CGH) study of PT has been published [11]. Our aim in this study was to identify DNA copy number changes that may lead to useful insights into the tumorigenesis of PT and to determine whether specific genetic alterations are associated with particular histological categories. In addition, we analysed allelic imbalance (AI) on 3p, which has been postulated to be the most common chromosomal abnormality in PT [11–13].

## 2. Materials and methods

### 2.1. Comparative genomic hybridization

CGH was performed on 22 paraffin-embedded tumour specimens from patients with PT of the breast. Table 1 shows the histopathological and clinical data of our patients. The histological diagnoses were performed in the Department of Pathology, ASAN Medical Center (Seoul, South Korea) according to the AFIP

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Table 1

Summary of clinical and pathological data, and comparative genomic hybridization (CGH) results from 22 patients with phyllodes tumour of the breast

| Type       | Patient Nos. | Age (years) | Tumour size (cm)        | Follow-up (months) | Local recurrence | CGH results           |                      |
|------------|--------------|-------------|-------------------------|--------------------|------------------|-----------------------|----------------------|
|            |              |             |                         |                    |                  | Gains                 | Losses               |
| Benign     | 1            | 29          | 3.5 × 3 × 1             | 14                 | no               | 1q21–23               | 6q13–q23             |
|            | 2            | 49          | 4 × 4 × 4               | 26                 | no               | 1q                    | –                    |
|            | 3            | 28          | 11 × 9 × 4              | 22                 | no               | 1q, 10                | 1p21–p32             |
|            | 4            | 51          | 7.5 × 4.5 × 2           | 40                 | no               | 1q21–qter, 17, 19, 20 | 2, 6q, 13, 14q11–q24 |
|            | 5            | 47          | 3 × 3 × 3               | 16                 | no               |                       | Normal               |
|            | 6            | 48          | 4 × 2 × 1.8             | 23                 | yes              |                       | Normal               |
|            | 7            | 32          | 3 × 2.5 × 1.5           | 19                 | no               |                       | Normal               |
|            | 8            | 58          | 4 × 3.5 × 2.5           | 23                 | no               | 1q                    | –                    |
|            | 9            | 48          | 4.5 × 4 × 2.5           | 25                 | no               | 1q                    | –                    |
| Borderline | 10           | 49          | 3 × 2 × 2               | 28                 | no               | 1q21–qter             | –                    |
|            | 11           | 47          | 11 × 10 × 7             | 38                 | no               |                       | Normal               |
|            | 12           | 49          | 4 × 4 × 4               | 53                 | no               |                       | Normal               |
|            | 13           | 32          | 1.5 × 1.5 × 1.5         | 30                 | no               | 1q21–q23              | –                    |
|            | 14           | 63          | 3 × 2.5 × 1.5           | 24                 | no               | 1q                    | 6q, 13               |
|            | 15           | 43          | 1 × 1 × 1               | 3                  | yes              |                       | Normal               |
| Malignant  | 16           | 32          | 19.5 × 12 × 11          | 32                 | no               | –                     | 9p22–pter            |
|            | 17           | 52          | 5 × 5 × 4               | 18                 | no               | 1q, 16p               | 4q23–q32, 13q11–q31  |
|            | 18           | 49          | 3.7 × 3.1 × 2.5         | 19                 | no               |                       | Normal               |
|            | 19           | 30          | 2.8 × 2.3 × 2           | 21                 | no               | 1q, 4p, 16, 17q       | 5q13–q31             |
|            | 20           | 33          | 5.5 × 4.5 and 3.7 × 2.2 | 19                 | no               | 1q                    | –                    |
|            | 21           | 63          | 14 × 10.5 × 7           | 7                  | no               | 1q                    | –                    |
|            | 22           | 28          | 12 × 9 × 6              | 5                  | no               |                       | Normal               |

criteria [14]. Our study followed the AFIP criteria: for example, a sample was considered malignant when stromal hypercellularity accompanied prominent mitotic activity exceeding five mitoses per ten high-power fields. Patient history and follow-up data were obtained by chart review and by information provided by the referring pathologist (GG) and surgeon (ASH, PJM). None of patients we studied died of this disease. Tumour area with more than 80% of tumour cells was selected from hematoxylin and eosin (H&E)-stained slides of each case and new paraffin-embedded blocks were made from the selected tumours. DNA was extracted from the new blocks as previously described [15]. CGH was performed as described in our previous reports [15,16]. In each CGH experiment, a negative (peripheral blood DNA from normal controls) and a positive (tumour DNA with known copy number changes) control were included and run simultaneously with the tumour samples. CGH images were analysed using the ISIS analysis system (MetaSystems GmbH, Altussheim, Germany). Based on our earlier reports and control results, we used 1.17 and 0.85 as

cut-off levels for gains and losses, respectively. All results were confirmed using 99% confidence interval.

## 2.2. Allelic imbalance (AI)

We used LOH analysis to detect allelic imbalance on 3p. Three microsatellite markers (D3S1300, D3S1478 and D3S1293) were selected (see <http://www.chlc.org>). DNA samples from the tumour and a normal counterpart of the same tissue were amplified by PCR reactions. The reaction was done in a 20 µl volume containing 50 ng DNA, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 10 mM Tris-HCl (pH 8.3), 0.1% Triton, 1% SDS, 0.2 mM of each dNTPs and 0.8 Unit Tag DNA polymerase (Promega, Southampton, UK). The amplification program consisted of 40 cycles at 94°C for 30 seconds, 1 minute at the appropriate annealing temperature (55–65°C), 15 seconds at 72°C and 6 minutes at 72°C after the last cycle.

The PCR results were evaluated after gel electrophoresis (6% polyacrylamide) and exposed to a film (Kodak XAR). The imbalance was interpreted visually

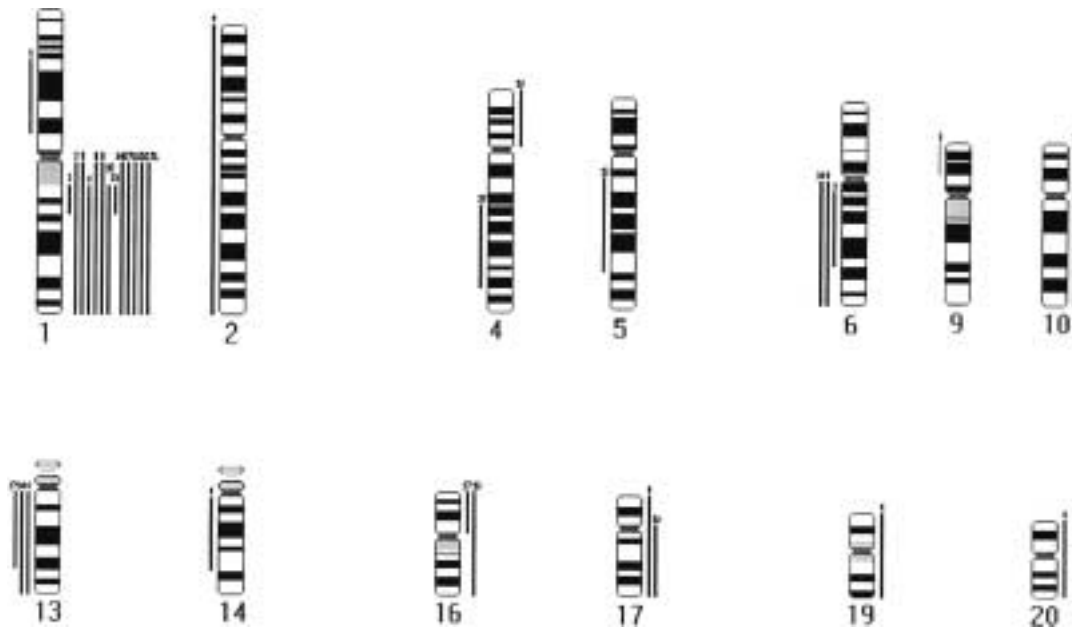


Fig. 1. Selected comparative genomic hybridization profiles of all losses and gains of DNA sequences observed in 22 phyllodes tumour specimens. Each chromosome is schematically depicted with areas of DNA loss represented by bars to the left and areas of DNA gain by bars to the right of the chromosome ideogram.

by comparing the intensity between the alleles in tumour DNA to those in normal DNA. Tumours homozygous for a specific locus were defined as uninformative. AI was detected from heterozygous samples when one of two polymorphic alleles in the tumour was either increased or reduced in intensity relative to the remaining allele and those in the corresponding normal-tissue DNA. All reactions were repeated and the results were confirmed.

### 3. Results

Colour figure can be viewed on <http://www.esacp.org/acp/2003/25-2/jee.htm>.

DNA copy number changes were detected in 14 tumours (63%). The average number of changes per tumour was 1.5. The most common abnormality was gain of 1q. It was detected in 12 cases and its locus was restricted to 1q21–24 in two cases (Fig. 1). No high-level amplifications were seen. There was no association with known parameters, such as the histopathological pattern, size and recurrence of the tumours. Table 1 shows the results in detail. Gains in 4p, 10, 16, 17 and 20 and losses of 1p, 2, 4q, 5q, 6q, 9p, 13 and 14q were observed in at least one of the cases.

Of the 20 tumours studied for AI, nine were informative at the D3S1300 locus (3p14.2), 13 at D3S1478

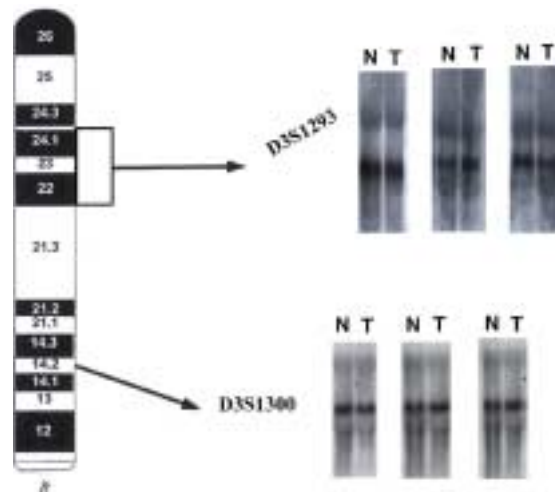


Fig. 2. Chromosome 3p allelic imbalance analysis using microsatellite markers. Results in paired normal and tumour samples. This figure can be viewed on <http://www.esacp.org/acp/2003/25-2/jee.htm>.

(3p21.3) and 16 at D3S1293 (3p22–24). No allelic imbalance was found in any of the tumours (Fig. 2).

### 4. Discussion

Only one previous CGH study of phyllodes tumours has shown gain in 1q and loss on 3p to be the two

most common chromosomal abnormalities [11]. The gain in 1q was observed to be recurrent (6 out of 21 patients) and it was shown to be associated with more aggressive tumour behaviour [11]. We could not find any differences between the presence of 1q and the histologic subtype (benign, borderline or malignant). Although 1q gain has not been specifically associated with phyllodes tumours, it has been reported to be present in a great variety of human malignancies [17]. It is a frequent change also in breast carcinomas. The recurrence of 1q gain as the sole abnormality suggests that it is an early event in PT [18]. Possible candidate genes in the minimum overlapping area of the 1q region (1q21–23) are *NTRK1* (neurotrophic tyrosine kinase receptor type 1), *APOA2* (apolipoprotein A), *FCHL* (hyperlipidemia) and *MUC1* (mucin1). Apart from *MUC1*, the role of other genes in PT is still an open question. *MUC1* has been reported to be aberrantly expressed in breast cancer [19].

Loss of heterozygosity or allelic imbalance involving polymorphic markers on the short arm of chromosome 3 have previously been reported to be frequent in phyllodes tumours [9,12]. However, we could not confirm the copy number changes on 3p by CGH nor the allelic imbalance in 3p (3 loci) by LOH analysis. Given that we studied only Korean patients, it is relevant to question whether ethnic differences could account for the discrepancy between our findings and earlier reports. Another consideration is the possibility of artifacts that LOH may show because the amplification strategies of GC-rich regions and AT-rich regions differ in PCR.

In conclusion, the gain in 1q, most probably within the area of 1q21–23, is important for the tumorigenesis of phyllodes tumour. Additional research is needed to identify the target genes in 1q. The final answer to the question whether LOH is present on 3p needs further studies.

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