
Allelic Imbalance in Primary Breast Carcinomas and Metastatic Tumors of the Axillary Lymph Nodes

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Abstract

Axillary lymph node status is the most important prognostic factor in predicting disease outcome in women with breast cancer. A number of chromosomal aberrations in primary breast tumors have been correlated with lymph node status and clinical outcome, but chromosomal changes particular to metastatic lymph node tumors have not been well studied. DNA samples isolated from laser-microdissected primary breast and metastatic axillary lymph node tumors from 25 women with invasive breast cancer were amplified using 52 microsatellite markers defining 26 chromosomal regions commonly deleted in breast cancer. Levels and patterns of allelic imbalance (AI) within and between breast and lymph node tumors were assessed to identify chromosomal alterations unique to primary or metastatic tumors and to examine the timing of metastatic potential. The overall frequency of AI in primary breast tumors (0.24) was significantly greater ($P < 0.001$) than that in lymph node tumors (0.10), and congruent AI events were observed for <20% of informative markers. AI at chromosomes 11q23.3 and 17p13.3 occurred significantly more frequently ($P < 0.05$) in primary breast tumors alone; no chromosomal regions showed a significantly higher AI frequency in lymph nodes. Higher rates of AI in primary versus metastatic lymph node tumors suggest that acquisition of metastatic potential may be an early event in carcinogenesis, occurring before significant levels of AI accumulate in the primary tumor. In addition, patterns of AI were highly discordant between tumor types, suggesting that additional genetic alterations accumulated independently in the two cell populations. (Mol Cancer Res 2005;3(2):71–7)

Introduction

A number of prognostic factors have been developed to guide treatment choices and predict clinical outcomes for breast cancer, including age, menopausal status, tumor stage, hormonal status, and lymph node involvement. Currently, metastasis to the axillary lymph nodes is the best known predictor of survival; individuals with negative lymph nodes have >90% 5-year survival compared with <70% survival in individuals with positive lymph nodes (1).

Development of a metastatic phenotype requires multiple steps including invasion, angiogenesis, intravasation, extravasation, and metastatic growth (2, 3). Although the model of clonal evolution in which cells acquire metastatic potential late in the development of the primary tumor, describes the progression of colorectal tumors, recent studies suggest that metastatic potential in breast cancer may be acquired early in the carcinogenic process. Although some investigators have shown that patterns of gene expression and allelic imbalance (AI) in primary breast tumors are similar to those in the corresponding metastases (4, 5), others have found disparate patterns of genetic change in primary breast tumors and distant/bone metastases (6, 7). Thus, it is unclear whether cells develop the ability to metastasize with relatively few genetic alterations early in tumor development or whether full metastatic potential is attained after additional genetic changes accumulate in disseminated breast cells.

To identify genetic alterations associated with the timing and acquisition of metastatic potential, we have used a panel of 52 microsatellite markers representing 26 chromosomal regions commonly deleted in breast cancer to identify patterns of AI in primary breast tumors and matched metastatic tumors of the axillary lymph nodes. Our objectives were to examine levels and patterns of chromosomal change both within and between primary breast and lymph node tumors to (a) assess levels of AI commonly associated with lymph node metastasis and (b) identify specific chromosomal regions associated with metastatic potential.

Results

Clinicopathologic Features

All samples were collected from female patients with an average age at diagnosis of 61.7 years; 28% (7 of 25) of patients were premenopausal (<50 years of age). The majority of cases (17 of 25) were diagnosed as invasive ductal carcinoma and represent stages IIA to IIIC. Average disease-free survival time was 40.6 months and 80% of patients are currently living with no evidence of disease.

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AI in Primary Breast Tumors and Metastatic Lymph Node Tumors

To increase the ability to detect AI within a given chromosomal region, two markers flanking each region were used. All markers had published heterozygosity values of >0.60, although within this data set, heterozygosity ranged from 0.32 (D3S2454) to 0.96 (D7S490). Observed heterozygosities were often lower than published values, but evaluation of each chromosomal region by marker pairs (Table 1) greatly improved informativeness. Five individual markers (D2S442, D16S2624, D16S516, D16S520, and D17S1298) showed AI in >50% of patients. When stratified into primary breast tumor versus lymph node tumor, these five markers showed rates of AI of >40% in the primary breast tumor, whereas the highest frequency of AI in the lymph node tumors occurred at D2S442 for 27% of patients. Analysis of the data by chromosomal region showed that 33% of patients showed AI at chromosomes 2q21-q23, 11q23.3, 16q11.2-q22.1, 16q22.3-q24.3, 17p13.1, and 17p13.3. Rates of AI ranged from 4% at chromosome 7q31 to 57% at chromosomes 16q22.3-q24.3 and 17p13.3. In comparisons of AI in breast versus lymph node tumor, the most frequent AI was seen at chromosomes 11q23.3 (45%), 16q11.2-q22.1 (45%), 16q22.3-q24.3 (52%) and 17p13.3 (48%), and at chromosomes 11q13.4 (25%) and 16q22.3-q24.3 (20%), respectively (Table 1).

Heterogeneity of AI between Primary Breast Tumors and Metastatic Lymph Node Tumors

Rates of discordance between primary breast and lymph node tumors measured as the number of noncongruent AI events over the total number of informative events (by chromosomal region), ranged from 0.05 to 0.59 with an average value of 25% discordance (Fig. 1). The overall frequency of AI in primary breast tumors (146 of 605) was significantly higher than that in metastatic lymph node tumors (63 of 602; $P < 0.001$). When AI frequencies for each chromosomal region were considered, rates of AI were significantly higher in primary breast tumors for chromosomes 11q23.3 and 17p13.3. Only chromosome 14q32.11-q31 showed a higher frequency of AI in the lymph nodes than in the primary breast tumor, although this difference was not significant. When the markers were examined individually, only D17S1298 on chromosome 17p13.3, showed significantly more frequent AI ($P < 0.03$) in primary breast tumors compared with metastatic lymph node tumors. Although the second marker from 17p13.3 (D17S849) showed high levels of AI, these differences did not reach statistical significance ($P = 0.0801$), suggesting a chromosomal region centromeric to D17S849 is involved in tumorigenesis.

Stratification of the AI by breast tumor only, metastatic lymph node tumor only, or both tumor types (congruent AI), revealed that AI unique to primary breast tumors comprised the majority of AI events (>60%), whereas both AI unique to the lymph node tumors and that seen congruently were less frequent (Fig. 2). When the data were examined by chromosomal region, congruent AI at chromosome 11q13.1 was more frequent than unique AI in either tumor type.

To confirm that congruent AI events represented true ancestral (shared) versus independently derived events, we

examined whether the same allele was altered in both the breast and lymph node. Overall, 91% of the shared AI events involved the same allele, supporting the hypothesis that these genomic changes occurred in the primary tumor before departure of metastatic cells.

Based on the patterns of unique and congruent AI events, a model depicting regions that likely play key roles in early and later stages of tumor development is shown in Fig. 3. Six chromosomal regions showed congruent rates of AI of >25% suggesting that these regions may be altered early in the process of tumorigenesis and may contribute to the acquisition of metastatic potential. Conversely, eight chromosomal regions showed high rates (>70%) of unique AI events in the breast, suggesting that alterations of these regions occur in later stages of tumor development, after cells with metastatic potential have dispersed.

Quality Control

The small quantities of DNA isolated after laser microdissection of paraffin-embedded samples precluded replication of all genotype data. Confirmatory analyses, done when AI was observed at only one marker for a given region using DNA isolated from the last serial sections, confirmed AI of ~80% of the time. Genotypes for which AI was not confirmed may reflect PCR variability or intratumoral heterogeneity (8-10).

Discussion

Traditional models of carcinogenesis describe tumor progression as being driven by the sequential accumulation of genetic alterations, with tumors amassing a critical assortment of genomic changes during development of invasive and metastatic capabilities (11). Accordingly, cells that acquire the ability to metastasize have a selective growth advantage and become the predominant cell type in the primary tumor. Whereas this model of clonal evolution may describe molecular events characteristic of colorectal cancer, recent studies have shown that for a number of human cancers, breast included, cells with metastatic potential may not predominate and dissemination of these cells to distant sites may occur early in tumor development (6, 12, 13).

Because chromosomal alterations are generally irreversible, genetic changes occurring in precursor cells should also be present in all progeny; therefore, metastatic cells should share many of the genetic abnormalities found in the primary tumor. In this study, overall frequencies of AI were significantly greater in primary breast than in metastatic lymph node tumors, suggesting that (a) metastatic tumor cells diverged early in the primary tumor development with relatively few genetic alterations, and (b) divergent patterns of genetic alterations accumulated independently in the two cell populations after metastatic cells departed from the primary breast carcinoma.

Examination of the chromosomal regions examined here (Fig. 3) also support the theory that metastatic potential may originate early in tumor development. AI at chromosomes such as 6q25-q27, 11q13.1, 13q12, and 16q22.1 has been detected in preinvasive lesions such as ductal carcinoma *in situ* and atypical ductal hyperplasia (14-17) and associated with early tumor development. On the other hand, AI for chromosomal

Table 1. Marker Location, Informativeness, and Frequency of AI

CDR	Marker Name	Marker Location	Marker Heterozygosity	Pairwise informative rate*	Frequency loss, primary breast tumor	Frequency loss, lymph node metastases																																																																																																																																																																																																																																																						
1p36.1-p36.2	D1S468	1p36.32	0.88	0.96	0.17 (4/24)	0.13 (3/24)																																																																																																																																																																																																																																																						
	D1S1612	1p36.23	0.72				2q21.3-23.3	D2S442	2q21.3	0.64	0.92	0.26 (6/23)	0.13 (3/23)	D2S1353	2q24.1	0.84	3p14.1	D3S1600	3p14.2	0.88	0.88	0.09 (2/22)	0.05 (1/22)	D3S2454	3p13	0.32	5q21.1-q21.3	D5S1721	5q21.2	0.74	0.96	0.13 (3/24)	0.04 (1/23)	D5S1478	5q23.1	0.92	6q15	D6S1043	6q16.1	0.79	0.92	0.17 (4/23)	0.09 (2/23)	D6S300	6q16.1	0.72	6q22.1-q23.1	D6S474	6q21	0.84	0.92	0.22 (5/23)	0.09 (2/23)	D6S242	6q22.3	0.80	6q25.2-q27	D6S441	6q25.2	0.92	1.00	0.36 (9/25)	0.16 (4/25)	D6S1027	6q27	0.92	7q31.1-q31.31	D7S523	7q31.1	0.80	1.00	0.04 (1/25)	0.00 (0/25)	D7S490	7q31.32	0.96	8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)	D8S511	8p22	0.72	8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)
2q21.3-23.3	D2S442	2q21.3	0.64	0.92	0.26 (6/23)	0.13 (3/23)																																																																																																																																																																																																																																																						
	D2S1353	2q24.1	0.84				3p14.1	D3S1600	3p14.2	0.88	0.88	0.09 (2/22)	0.05 (1/22)	D3S2454	3p13	0.32	5q21.1-q21.3	D5S1721	5q21.2	0.74	0.96	0.13 (3/24)	0.04 (1/23)	D5S1478	5q23.1	0.92	6q15	D6S1043	6q16.1	0.79	0.92	0.17 (4/23)	0.09 (2/23)	D6S300	6q16.1	0.72	6q22.1-q23.1	D6S474	6q21	0.84	0.92	0.22 (5/23)	0.09 (2/23)	D6S242	6q22.3	0.80	6q25.2-q27	D6S441	6q25.2	0.92	1.00	0.36 (9/25)	0.16 (4/25)	D6S1027	6q27	0.92	7q31.1-q31.31	D7S523	7q31.1	0.80	1.00	0.04 (1/25)	0.00 (0/25)	D7S490	7q31.32	0.96	8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)	D8S511	8p22	0.72	8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88						
3p14.1	D3S1600	3p14.2	0.88	0.88	0.09 (2/22)	0.05 (1/22)																																																																																																																																																																																																																																																						
	D3S2454	3p13	0.32				5q21.1-q21.3	D5S1721	5q21.2	0.74	0.96	0.13 (3/24)	0.04 (1/23)	D5S1478	5q23.1	0.92	6q15	D6S1043	6q16.1	0.79	0.92	0.17 (4/23)	0.09 (2/23)	D6S300	6q16.1	0.72	6q22.1-q23.1	D6S474	6q21	0.84	0.92	0.22 (5/23)	0.09 (2/23)	D6S242	6q22.3	0.80	6q25.2-q27	D6S441	6q25.2	0.92	1.00	0.36 (9/25)	0.16 (4/25)	D6S1027	6q27	0.92	7q31.1-q31.31	D7S523	7q31.1	0.80	1.00	0.04 (1/25)	0.00 (0/25)	D7S490	7q31.32	0.96	8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)	D8S511	8p22	0.72	8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																
5q21.1-q21.3	D5S1721	5q21.2	0.74	0.96	0.13 (3/24)	0.04 (1/23)																																																																																																																																																																																																																																																						
	D5S1478	5q23.1	0.92				6q15	D6S1043	6q16.1	0.79	0.92	0.17 (4/23)	0.09 (2/23)	D6S300	6q16.1	0.72	6q22.1-q23.1	D6S474	6q21	0.84	0.92	0.22 (5/23)	0.09 (2/23)	D6S242	6q22.3	0.80	6q25.2-q27	D6S441	6q25.2	0.92	1.00	0.36 (9/25)	0.16 (4/25)	D6S1027	6q27	0.92	7q31.1-q31.31	D7S523	7q31.1	0.80	1.00	0.04 (1/25)	0.00 (0/25)	D7S490	7q31.32	0.96	8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)	D8S511	8p22	0.72	8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																										
6q15	D6S1043	6q16.1	0.79	0.92	0.17 (4/23)	0.09 (2/23)																																																																																																																																																																																																																																																						
	D6S300	6q16.1	0.72				6q22.1-q23.1	D6S474	6q21	0.84	0.92	0.22 (5/23)	0.09 (2/23)	D6S242	6q22.3	0.80	6q25.2-q27	D6S441	6q25.2	0.92	1.00	0.36 (9/25)	0.16 (4/25)	D6S1027	6q27	0.92	7q31.1-q31.31	D7S523	7q31.1	0.80	1.00	0.04 (1/25)	0.00 (0/25)	D7S490	7q31.32	0.96	8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)	D8S511	8p22	0.72	8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																				
6q22.1-q23.1	D6S474	6q21	0.84	0.92	0.22 (5/23)	0.09 (2/23)																																																																																																																																																																																																																																																						
	D6S242	6q22.3	0.80				6q25.2-q27	D6S441	6q25.2	0.92	1.00	0.36 (9/25)	0.16 (4/25)	D6S1027	6q27	0.92	7q31.1-q31.31	D7S523	7q31.1	0.80	1.00	0.04 (1/25)	0.00 (0/25)	D7S490	7q31.32	0.96	8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)	D8S511	8p22	0.72	8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																														
6q25.2-q27	D6S441	6q25.2	0.92	1.00	0.36 (9/25)	0.16 (4/25)																																																																																																																																																																																																																																																						
	D6S1027	6q27	0.92				7q31.1-q31.31	D7S523	7q31.1	0.80	1.00	0.04 (1/25)	0.00 (0/25)	D7S490	7q31.32	0.96	8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)	D8S511	8p22	0.72	8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																								
7q31.1-q31.31	D7S523	7q31.1	0.80	1.00	0.04 (1/25)	0.00 (0/25)																																																																																																																																																																																																																																																						
	D7S490	7q31.32	0.96				8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)	D8S511	8p22	0.72	8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																		
8p22-p21.3	D8S552	8p22	0.88	0.88	0.14 (3/22)	0.14 (3/22)																																																																																																																																																																																																																																																						
	D8S511	8p22	0.72				8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)	D8S284	8q24.21	0.75	9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																												
8q24	D8S514	8q24.13	0.76	0.92	0.26 (6/23)	0.09 (2/23)																																																																																																																																																																																																																																																						
	D8S284	8q24.21	0.75				9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)	D9S304	9p21.1	0.76	10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																						
9p21	D9S171	9p21.3	0.84	0.96	0.13 (3/24)	0.08 (2/24)																																																																																																																																																																																																																																																						
	D9S304	9p21.1	0.76				10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)	D10S677	10q23.33	0.68	11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																
10q23.31-q23.33	D10S541	10q23.31	0.71	0.96	0.29 (7/24)	0.13 (3/23)																																																																																																																																																																																																																																																						
	D10S677	10q23.33	0.68				11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)	D11S2368	11p15.1	0.75	11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																										
11p15	D11S1999	11p15.3	0.76	0.88	0.27 (6/22)	0.05 (1/22)																																																																																																																																																																																																																																																						
	D11S2368	11p15.1	0.75				11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)	D11S901	11q14.1	0.88	11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																				
11q13.1	D11S2371	11q13.4	0.68	0.96	0.25 (6/24)	0.25 (6/24)																																																																																																																																																																																																																																																						
	D11S901	11q14.1	0.88				11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)	D11S925	11q23.3	0.72	13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																														
11q23.3	D11S4464	11q24.1	0.74	0.84	0.45 (9/20)	0.14 (3/21)																																																																																																																																																																																																																																																						
	D11S925	11q23.3	0.72				13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)	D13S289	13q12.3	0.48	13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																								
13q12.3	D13S1246	13q12.3	0.84	0.96	0.25 (6/24)	0.08 (2/24)																																																																																																																																																																																																																																																						
	D13S289	13q12.3	0.48				13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)	D13S155	13q14.3	0.84	14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																		
13q14.2-q14.3	D13S153	13q14.2	0.88	0.96	0.25 (6/24)	0.17 (4/24)																																																																																																																																																																																																																																																						
	D13S155	13q14.3	0.84				14q32.11-q31	D14S1279	14q31.3	0.68	0.96	0.04 (1/24)	0.08 (2/24)	D14S617	14q32.12	0.71	16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																												
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	D14S617	14q32.12	0.71				16q11.2-q22.1	D16S409	16q11.2	0.68	0.88	0.45 (10/22)	0.18 (4/22)	D16S2624	16q22.2	0.68	16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																																						
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	D16S2624	16q22.2	0.68				16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)	D16S520	16q24.1	0.52	17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																																																
16q22.3-q24.3	D16S516	16q23.2	0.72	0.84	0.52 (11/21)	0.20 (4/20)																																																																																																																																																																																																																																																						
	D16S520	16q24.1	0.52				17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)	D17S1298	17p13.3	0.65	17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																																																										
17p13.3	D17S849	17p13.3	0.64	0.84	0.48 (10/21)	0.16 (3/19)																																																																																																																																																																																																																																																						
	D17S1298	17p13.3	0.65				17p13.1	D17S938	17p13.2	0.83	0.96	0.38 (9/24)	0.13 (3/24)	D17S786	17p13.1	0.72	17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																																																																				
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	D17S786	17p13.1	0.72				17q21.23	D17S250	17q12	0.76	1.00	0.24 (6/25)	0.04 (1/25)	D17S579	17q21.31	0.92	18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																																																																														
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	D17S579	17q21.31	0.92				18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)	D18S64	18q21.32	0.68	22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																																																																																								
18q21.1-q21.3	D18S548	18q12.3	0.76	0.88	0.14 (3/22)	0.09 (2/22)																																																																																																																																																																																																																																																						
	D18S64	18q21.32	0.68				22q12.3	D22S281	22q12.3	0.92	1.00	0.20 (5/25)	0.12 (3/25)	D22S277	22q12.3	0.91	22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																																																																																																		
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	D22S277	22q12.3	0.91				22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)	D22S423	22q13.1	0.88																																																																																																																																																																																																																																												
22q13.1	D22S1045	22q13.1	0.80	1.00	0.20 (5/25)	0.04 (1/25)																																																																																																																																																																																																																																																						
	D22S423	22q13.1	0.88																																																																																																																																																																																																																																																									

*Values combine data from markers for a given chromosomal region.

regions such as 8q24, 11p15, 11q23.3, 17p13.3, 17p13.1, and 17q21 has been associated with an invasive phenotype, tumor progression, and poor prognosis (18-21), findings consistent with our data showing higher frequency of AI at these regions only in primary breast tumors. In addition, a number of recent studies using genomic technologies such as AI analysis, comparative genomic hybridization, and gene expression analysis to examine genetic characteristics of primary breast and metastatic tumors have developed models suggesting that metastatic potential is acquired early in tumor development (4, 6, 7, 22). Similar models have been proposed for non-small cell lung carcinoma and melanoma, as well (13, 23).

Many recent publications have proposed that acquisition of metastatic potential is an early-stage event; however, there is debate about whether metastasis-specific genetic alterations exist. For example, Bonsing et al. (4) examined patterns of AI in diploid and aneuploid cells of primary breast and matched metastatic lymph node tumors. Although the rate of AI was increased in aneuploid cells of metastatic lymph nodes, the presence of diploid precursor clones in the metastatic tumors led them to conclude that additional genetic alterations are not needed for metastatic potential. Likewise, microarray studies showing similar gene expression patterns between primary breast tumors and metastases (5) seem to support the hypothesis that exclusive genetic changes are not required for metastasis (22).

Conversely, discordant patterns of genetic change between primary breast tumors and distant metastases detected using comparative genomic hybridization suggest that metastatic cells may acquire a growth or survival-promoting array of alterations after dissemination (6, 7). In accordance with these studies, our observations of highly discordant patterns of AI between the primary breast and metastatic lymph node tumors supports the independent evolution of genomic alterations in the two tumor types and suggest that patterns of AI in the primary tumor may not be prognostic of metastatic tumor development and behavior.

In the data presented here, patterns of AI in the lymph node metastases were less complex than the primary tumors. In the study by Bonsing et al. (4), sorting of diploid and aneuploid cells allowed, in some cases, a more accurate detection of AI. Because we did not sort cells by karyotype, it is possible that rates of AI are underrepresented in our data set, although this underrepresentation should also be present in the primary tumors. To examine whether low levels of AI in the metastatic tumors reflected a sampling artifact, three patients with multiple positive nodes were identified and tumor cells from each node were microdissected and subjected to AI analysis. The number of microdissected nodes ranged from two to seven, and in all cases, the frequency of AI was lower in the lymph node tumors than in the primary breast tumors, suggesting that the lower frequency of AI in the lymph node tumor was not reflective of the specific node dissected.

Patterns of AI consistent with a metastatic phenotype were not observed in this study, which may be due to the low levels of alterations in the lymph nodes detected with this marker panel. Twenty-six chromosomal regions commonly deleted in breast cancer (24) were examined; these regions which are important to primary breast cancer development may not be relevant to the acquisition of full metastatic capabilities. It is, therefore, possible that an independent set of chromosomal alterations, not assayed here, is critical to metastatic tumor growth and survival. Whereas both primary and metastatic tumors share a number of common features, such as uncontrolled proliferation and escape from cell cycle checkpoints, metastatic tumors represent specific cell types transplanted to a distant tissue microenvironment, with unique

challenges to survival and growth. In addition, metastatic tumor growth and propagation may follow pathways quite different from that of the primary tumor (6, 7). The use of a global profiling approach may identify a set of chromosomal alterations important for survival and growth of metastatic lymph node tumors.

In conclusion, AI data from primary breast and metastatic lymph node tumors support an emerging model of acquisition of metastatic potential early in the development of the primary tumor. In contrast with other studies that observed similar or higher levels of genomic instability in breast metastases, the discordant patterns of genetic alterations detected by AI supports a model in which additional genetic alterations may be necessary for disseminated breast cells to reach their full metastatic potential. As many genomic changes in the primary tumor may evolve independently after metastatic cells have departed, genetic profiling of the primary tumor may not adequately predict metastatic behavior. The application of global profiling technologies may identify specific molecular alterations that confer upon metastatic breast cells the ability to thrive in a foreign environment, and allow for development of novel diagnostics targeted to metastatic tumors.

Materials and Methods

Paraffin-embedded primary breast and matched axillary lymph node tumor tissues from 25 patients were obtained from the Windber Medical Center Pathology Department or the Clinical Breast Care Project (CBCP) Pathology Laboratory. Samples from the Windber Medical Center ($n = 8$) were archival in nature and anonymized with no links between the assigned research number and patient identifiers. Clinical information including age at diagnosis, estrogen receptor/progesterone receptor status, disease recurrence, and cause/date of death was provided anonymously by the Memorial Medical Center Cancer Registry. Tissue and blood samples from CBCP patients ($n = 17$) were collected with approval from the Walter Reed Army Medical Center Human Use Committee and Institutional Review Board. All subjects enrolled in the CBCP voluntarily agreed to participate and gave written informed

Sample #5	1p36	2q21-q23	3p14.1	5q21	6q15	6q22-q23	6q25-q27	7q31	8p22	8q24	9p21	10q23	11p15	11q13	11q23	13q12	13q14	14q31-q32	16q11-q22.1	16q22.3-q24	17p13.3	17p13.1	17q21	18q21	22q12	22q13	
PBT																											
MLN																											
Sample #9	1p36	2q21-q23	3p14.1	5q21	6q15	6q22-q23	6q25-q27	7q31	8p22	8q24	9p21	10q23	11p15	11q13	11q23	13q12	13q14	14q31-q32	16q11-q22.1	16q22.3-q24	17p13.3	17p13.1	17q21	18q21	22q12	22q13	
PBT																											
MLN																											

FIGURE 1. Patterns of AI in primary breast (PBT) and matched metastatic lymph node (MLN) tumors from two patients with stage IIIA invasive ductal carcinoma. *Black squares*, AI; *white squares*, normal chromosomal content. Patient 5 showed one of the highest rates of discordance (59%) in this data set. Patient 9 showed a similar overall AI frequency as patient 5; however, the frequency of discordant events was much lower (20%).

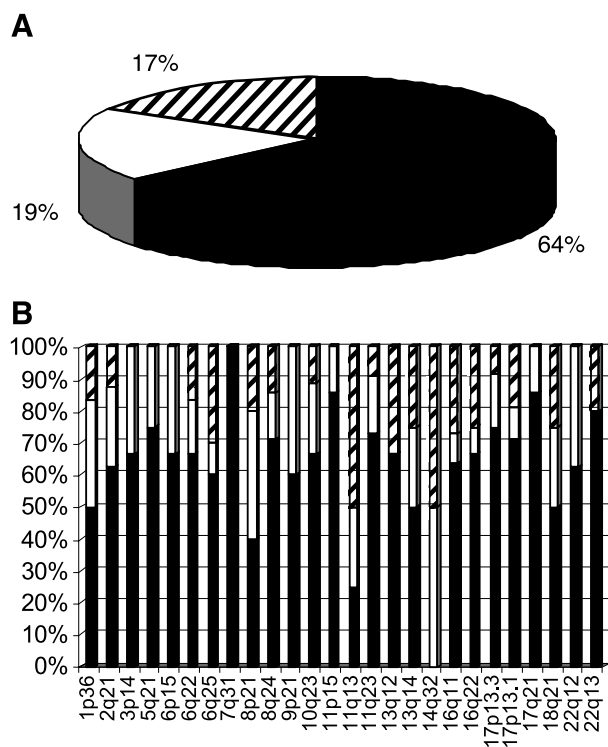


FIGURE 2. Frequency of unique and congruent AI between primary breast and metastatic lymph node tumors. The overall frequency of unique and congruent AI was calculated across all chromosomal regions (A) and then stratified by chromosomal region (B). PBT, primary breast tumor; MLN, metastatic lymph node tumor. ▨, Congruent; □, MLN; ■, PBT.

consent. Demographic and clinical information was provided for all CBCP samples using questionnaires designed by and given under the auspices of the CBCP. Diagnoses of all tumor samples were made by one pathologist from H&E-stained slides using the new guidelines defined by the *AJCC Cancer Staging Manual* (25). Specimens included stage IIA ($n = 6$), stage IIB ($n = 7$), stage IIIA ($n = 9$), and stage IIIC ($n = 3$) infiltrating ductal or lobular carcinomas. In addition,

five stage I to IIA primary breast tumors with negative lymph nodes were collected as control cases. Clinical information for all samples with positive lymph nodes is summarized in Table 2.

DNA was obtained from homogeneous populations of primary breast and metastatic axillary lymph node tumor cells following laser-assisted microdissection on an ASLMD laser microdissection system (Leica Microsystems, Wetzlar, Germany; ref. 26). All microdissected sections were examined by the CBCP pathologist who identified and marked regions of tumor before dissection. The integrity of multiple serial sections was established by pathologic verification of the first and last sections stained with H&E. Because small quantities of DNA isolated from formalin-fixed paraffin-embedded specimens have been associated with PCR artifacts (27), a minimum of 250 cells were used in every PCR reaction. To achieve this cell count, 5,000 cells were captured from each of six consecutive primary breast tumor sections, with the sixth section reserved for all confirmatory reruns. To achieve a similar cell count from the lymph nodes, cells were isolated and pooled from four consecutive sections, with sections 21 to 24 reserved for confirmations. Reference DNA samples for the archive samples were extracted from disease-free skin (nipple) tissue from each patient using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA). Reference DNA for the CBCP samples was obtained either from blood clots using Clotspin and Puregene DNA purification kits (Gentra, Minneapolis, MN), or from microdissected disease-free breast tissues.

Microsatellite markers (Table 1) were amplified as previously described (28), purified using Sephadex G-50 resin and genotyped on a MegaBACE-1000 capillary electrophoresis apparatus (Amersham Biosciences, Piscataway, NJ) following standard protocols. Genotypes were determined using Genetic Profiler version 2.0 software with alphabetical bin labels that facilitated accurate allele calling (Fig. 4). AI was detected using the formula $(T1 / T2) / (N1 / N2)$ where T1 and N1 represent the peak heights of the less intense alleles and T2 and N2 represent the peak heights of the more intense alleles of the tumor and referent samples, respectively (29).

Threshold values for determining AI in the literature vary widely from 20% to 75% change in tumor allele intensity

FIGURE 3. Genetic model of breast cancer progression. A model of tumor progression has been developed based on the unique or congruent frequency of AI in the primary breast tumor and metastatic lymph node tumors. Those regions with high rates of shared AI are considered early events in tumor progression, whereas those with high rates only in the PBT are thought to occur after the dispersal of cells with metastatic potential. Note that because chromosomal regions 7q31 and 14q31-q32 had AI frequencies <10%, these regions were not incorporated into the model.

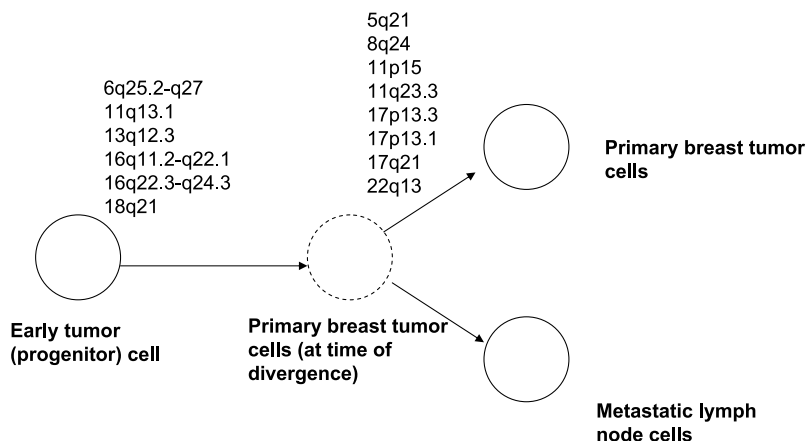


Table 2. Clinicopathologic Characteristics of 25 Primary Breast and Matched Metastatic Lymph Node Specimens

Patient no.	Age at Diagnosis	Histology	Tumor Size	No. Positive Lymph Nodes	TNM stage	Clinical Outcome	FAI* in Primary Tumor	FAI in Lymph Node Metastasis
1	40	IDCA	T2	2	IIB	ADF	0.48	0.09
2	67	IDCA	T1	1	IIA	DC	0.30	0.25
3	60	IDCA	T2	2	IIA	ADF	0.00	0.17
4	69	IDCA	T2	1	IIIB	ADF	0.04	0.38
5	77	ILCA	T3	4	IIIA	DC	0.59	0.18
6	81	IDCA	T3	1	IIIA	DO	0.17	0.14
7	61	IDCA	T2	1	IIB	ADF	0.58	0.00
8†	70	IDCA	T2	8	IIIA	DC	0.44	0.00
9	30	IDCA	T3	6	IIIA	ADF	0.32	0.44
10	60	ILCA	T2	4	IIIA	ADF	0.17	0.00
11	63	ILCA	T1c	4	IIIA	ADF	0.09	0.05
12	76	Mixed	T1b	1	IIA	ADF	0.25	0.25
13	48	ILCA	T2	2	IIB	ADF	0.24	0.00
14	58	IDCA	T2	1	IIB	ADF	0.31	0.00
15	65	ILCA	T1c	26	IIIC	AWD	0.12	0.04
16	73	IDCA	T2	1	IIB	ADF	0.16	0.09
17	68	IDCA	T1b	1	IIA	ADF	0.12	0.00
18	80	Mixed	T2	4	IIIA	ADF	0.33	0.25
19	52	IDCA	T1c	1	IIA	ADF	0.19	0.00
20	47	IDCA	T2	1	IIB	ADF	0.60	0.08
21	82	IDCA	T1c	1	IIA	ADF	0.13	0.13
22	46	IDCA	T1	7	IIIA	ADF	0.30	0.05
23	48	IDCA	T2	19	IIIC	ADF	0.04	0.04
24	48	IDCA	T2	10	IIIC	ADF	0.04	0.04
25	73	ILCA	T2	10	IIIA	ADF	0.12	0.00

NOTE: Mixed = invasive mammary carcinoma with mixed ductal and lobular features.

Abbreviations: IDCA, invasive ductal carcinoma; ILCA, invasive lobular carcinoma; ADF, alive disease-free; AWD, alive with disease; DC, deceased from cancer; DO, deceased, other; FAI, fractional allelic imbalance; TNM, tumor-node-metastasis.

*FAI calculated as the number of chromosomal regions showing AI divided by the number of informative genotypes.

†Samples 1 to 8, archival; samples 9 to 25, CBCP.

(27, 29-31). Data were analyzed using peak height ratios of <0.50 and <0.35 , and although <0.50 identified more AI events, the threshold value for detecting AI was set at 0.35 as this more stringent ratio was more robust to confirmatory analyses.

Each chromosomal region was represented by two polymorphic markers, permitting analysis by marker and by chromosomal region. When analyzed by region, AI was defined according to the following criteria: (a) when at least one marker for a given region showed an allelic ratio of <0.35 ,

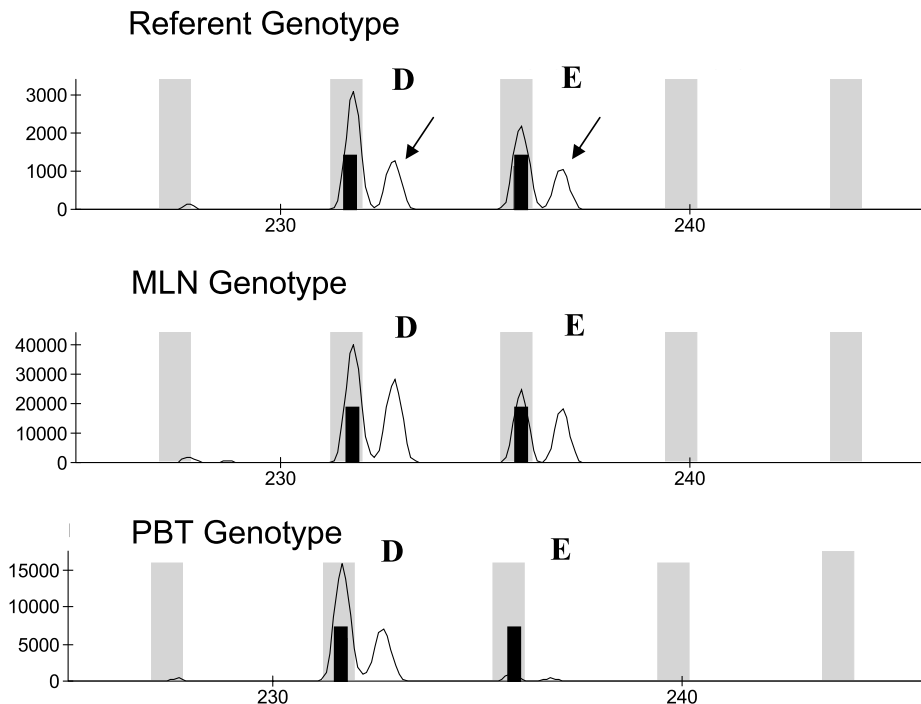


FIGURE 4. Detection of AI with fluorescence-based genotyping. Genotypes for referent (genomic) DNA, microdissected primary breast tumor (PBT) DNA and microdissected lymph node (MLN) tumor DNA for D11S4464 on chromosome 11q24.1 are shown (black columns) with each allele detected as a fluorescent peak. Stutter bands are marked with black arrows for the referent sample only. Alleles designated “D” and “E” correspond to molecular weight fragments of 232 and 236 base pairs, respectively. Peak height values were $D = 3,176$ rfu, $E = 2,248.3$ rfu (reference); $D = 40,228.3$ rfu, $E = 25,180.6$ rfu (MLN); and $D = 16,113.2$ rfu, $E = 1,098.4$ rfu (PBT). Using the ratio described in the Materials and Methods section, a normalized peak height ratio of 0.88 was calculated for the MLN and 0.10 was determined for the PBT, suggesting that the MLN shows no AI at 11q24.1, but the PBT has a deletion or amplification of this region.

the region was considered to show AI; (b) when neither marker had an allelic ratio of <0.35 and at least one marker was informative, the region was considered normal; and (3) when both markers were homozygous, the region was considered uninformative.

Comparison of the overall frequency of AI between breast and lymph node tumors was done using Student's *t* test; all other analyses used the Exact Unconditional Homogeneity/Independence Tests for 2×2 tables (<http://www4.stat.ncsu.edu/~berger/tables.html>). A significance value of $P < 0.05$ was used for all analyses.

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References

- National Cancer Institute. SEER cancer statistics review, 1973-1991. NIH Publ. No. 94-2789. Bethesda (MD): NIH; 1994.
- Nicolson GL. Molecular mechanisms of cancer metastasis: tumor and host properties and the role of oncogenes and suppressor genes. *Curr Opin Oncol* 1993;9:541-73.
- Stetler-Stevenson WG, Aznavoorian S, Liotta LA. Tumor cell interactions with the extracellular matrix during invasion and metastasis. *Annu Rev Cell Biol* 1993;9:541-73.
- Bonsing BA, Corver WE, Fleuren GJ, Cleton-Jansen AM, Devilee P, Cornelisse CJ. Allelotype analysis of flow-sorted breast cancer cells demonstrates genetically related diploid and aneuploid subpopulations in primary tumor and lymph node metastases. *Genes Chromosomes Cancer* 2002;28:183.
- van't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;415:530-6.
- Kuukasjarvi T, Karhu R, Tanner M, et al. Genetic heterogeneity and clonal evolution underlying development of asynchronous metastasis in human breast cancer. *Cancer Res* 1997;57:1597-604.
- Schmidt-Kittler O, Ragg T, Daskalakis A, et al. From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. *Proc Natl Acad Sci U S A* 2003;100:7737-42.
- Chen LC, Kurisu W, Ljung BM, Goldman ES, Moore DD, Smith HS. Heterogeneity for allelic loss in human breast cancer. *J Natl Cancer Inst* 1992;84:506-10.
- Hugel A, Wernert N. Loss of heterozygosity (LOH), malignancy by grade and clonality in microdissected prostate cancer. *Br J Cancer* 1999;79:551-7.
- Yatabe Y, Konishi H, Mitsudomi T, Nakamura S, Takahashi T. Topological distributions of allelic loss in individual non-small-cell lung cancers. *Am J Pathol* 2000;157:985-93.
- Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759-67.
- Bissig H, Richter J, Desper R, et al. Evaluation of the clonal relationship between primary and metastatic renal cell carcinoma by comparative genomic hybridization. *Am J Pathol* 1999;155:267-74.
- Sasatomi E, Finkelstein SD, Woods JD, et al. Comparison of accumulated allele loss between primary tumor and lymph node metastasis in stage II non-small cell lung carcinoma: implications for the timing of lymph node metastasis and prognostic value. *Cancer Res* 2002;62:2681-9.
- Amari M, Suzuki A, Monya T, et al. LOH analyses of premalignant and malignant lesions of human breast: frequent LOH in 8p, 16q, and 17q in atypical ductal hyperplasia. *Oncol Rep* 1999;6:1277-80.
- Chappell SA, Walsh T, Walker RA, Shaw JA. Loss of heterozygosity at chromosome 6q in preinvasive and early invasive breast carcinomas. *Br J Cancer* 1997;75:1324-9.
- Chuaqui RF, Zhuang Z, Emmert-Buck MR, Liotta LA, Merino MJ. Analysis of loss of heterozygosity on chromosome 11q13 in atypical ductal hyperplasia and *in situ* carcinoma of the breast. *Am J Pathol* 1997;150:297-303.
- Radford DM, Fair KL, Phillips TJ, et al. Allelotyping of ductal carcinoma *in situ* of the breast: deletion of loci on 8p, 13q, 16q, 17p and 17q. *Cancer Res* 1995;55:3399-405.
- Hampel M, Hampel JA, Schwarz P, et al. Accumulation of genetic alterations in brain metastases of sporadic breast carcinomas is associated with reduced survival after metastasis. *Invasion Metastasis* 1998;18:81-95.
- Karnik P, Paris M, Williams BRG, Casey G, Crowe J, Chen P. Two distinct tumor suppressor loci within chromosome 11p15 implicated in breast cancer progression and metastasis. *Hum Mol Genet* 1998;7:895-903.
- Martin ES, Cesari R, Pentimalli F, et al. The BCSC-1 locus at chromosome 11q23-q24 is a candidate tumor suppressor gene. *Proc Natl Acad Sci U S A* 2003;100:11517-22.
- Radford DM, Phillips NJ, Fair KL, et al. Allelic loss and the progression of breast cancer. *Cancer Res* 1995;55:5180-3.
- Bernards R, Weinberg RA. A progression puzzle. *Nature* 2002;418:823.
- Morita R, Fujimoto A, Hatta N, Takehara K, Takata M. Comparison of genetic profiles between primary melanomas and their metastases reveals genetic alterations and clonal evolution during progression. *J Invest Dermatol* 1998;111:919-24.
- Osborne RJ, Hamshire MG. A genome-wide map showing common regions of loss of heterozygosity/allelic imbalance in breast cancer. *Cancer Res* 2000;60:3706-12.
- American Joint Committee on Cancer. *AJCC cancer staging manual*, 6th ed. New York: Springer; 2002.
- Ellsworth DL, Shriver CD, Ellsworth RE, Deyarmin B, Somiari RI. Laser capture microdissection of paraffin-embedded tissues. *BioTechniques* 2003;34:42-6.
- Bertheau P, Plassa LF, Lerebours F, et al. Allelic loss detection in inflammatory breast cancer: improvement with laser microdissection. *Lab Invest* 2001;81:1397-402.
- Ellsworth RE, Ellsworth DL, Lubert SM, Hooke J, Somiari RI, Shriver CD. High-throughput loss of heterozygosity mapping in 26 commonly deleted regions in breast cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:915-9.
- Medintz IL, Lee C-CR, Wong WW, Pirkola K, Sidransky D, Mathies RA. Loss of heterozygosity assay for molecular detection of cancer using energy-transfer primers and capillary array electrophoresis. *Genome Res* 2000;10:1211-8.
- Shen C-Y, Yu J-C, Lo Y-L, et al. Genome-wide search for loss of heterozygosity using laser capture microdissected tissue of breast carcinoma: an implication for mutator phenotype and breast cancer pathogenesis. *Cancer Res* 2000;60:3884-92.
- Wang Y, Huang S-C, Linn JF, et al. Microsatellite-based cancer detection using capillary array electrophoresis and energy-transfer fluorescent primers. *Electrophoresis* 1997;18:1742-9.

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