Use of Monozygotic Twins in Search for Breast Cancer Susceptibility Loci

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We have used Swedish monozygotic twins concordant for breast cancer to study genetic changes associated with the development of breast cancer. Because loss of heterozygosity (LOH) at a specific genomic region may reflect the presence of a tumour suppressor gene, loss of the same allele in both of the twins concordant for breast cancer may pinpoint a tumour suppressor gene that confers a strong predisposition to breast cancer. DNA samples extracted from the matched tumour and normal tissues of nine twin pairs were analysed for allelic imbalance using a set of microsatellite markers on chromosomes 1, 13, 16 and 17, containing loci with known tumour suppressor genes. The two main regions, where more twin pairs than expected had lost the same allele, were located at $16q_{tel}$, including markers D16S393, D16S305 and D16S413, and at 17p13, distal to the p53 locus. Our results show that the monozygotic twin model can be used to suggest candidate regions of potential tumour suppressor genes, even with a limited number of twin pairs.

Monozygotic twins have rarely been used in molecular genetic studies because a twin pair is genetically identical at birth. However, the somatic events that ensue during the twins' lifetime may reveal the importance of inherited factors for disease causation. In cancer, one such event is loss of heterozygosity (LOH). Analysis of LOH has been an important approach in the search of new tumour suppressor genes. This is based on the paradigm first observed in retinoblastoma (Knudson, 1971), in which one normal allele of a tumour suppressor gene is inactivated by a mutation or a deletion, and in the process of cancer development it is converted to a homozygous or hemizygous form through a second independent alteration involving chromosomal nondisjunction and deletion, mitotic recombination, or gene conversion (Weinberg, 1991). If a locus were important for predisposition to cancer, concordant LOH with a loss of the same allele would be expected in tumour DNA of both twins because monozygotic twins share identical haplotypes. Due to heterogeneity of breast cancer different twin pairs would be expected to show LOH at different loci. The probability of monozygotic twins randomly losing the same allele at a locus is 50%. At a locus important for predisposition to cancer, a large number of twin pairs should have lost the same allele. Monozygotic twins concordant for cancer are rare but once available the model has a high statistical power to suggest candidate regions for potential tumour suppressor genes.

Breast cancer is a common disease in the western world, affecting one in ten women during their lifetime. Family history is a well-established and important risk factor for breast cancer. In a recent study on the Swedish, Danish and Finnish twins 27% of the breast cancer risk could be explained by hereditary factors (Lichtenstein et al., 2000). Analysis of high-risk breast cancer families with several affected family members has lead to the discovery of two genes predisposing to breast cancer, *BRCA1* and *BRCA2*, located on chromosomes 17 and 13, respectively (Hall et al., 1990; Miki et al., 1994; Tavtigian et al., 1996; Wooster et al., 1994; Wooster et al., 1995). However, mutations in these two high-risk genes only explain some 1–2% of the breast cancer cases (Peto et al., 1999; Syrjakoski et al., 2000). In our previous study on 12 Swedish monozygotic twin pairs concordant for breast cancer, two unclassified *BRCA2* variants, with a putative pathogenic effect, were identified, but no pathogenic alterations were found in the *BRCA1* gene (Försti et al., 2001). Taken together, the above data indicate that other inherited susceptibility genes with a more modest breast cancer risk are yet to be identified.

In this paper we extend our approach to study inherited changes associated with the development of breast cancer by analysing LOH in monozygotic twins concordant for breast cancer (Försti et al., 1997; Försti et al., 2001). Chromosomes 1 and 16 were included to the study in addition of chromosomes 13 and 17, which were analysed in our previous paper (Försti et al., 2001), and a thorough statistical analysis of hereditary effects in LOH was carried out. Our twin samples have been collected from breast cancers of Swedish twins that were included in the published epidemiological studies (Ahlbom et al., 1997; Lichtenstein et al., 2000). According to the latest Nordic study the risk of monozygotic twins for breast cancer was 5.2 and that of dizygotic twins 2.8 (Lichtenstein et al., 2000). The data imply that inherited factors are important determinants of breast cancer in monozygotic twins. In the present study we have analysed LOH on chromosomes 1, 13, 16 and 17, which belong to the chromosomes with most frequent LOH in breast cancer (Beckmann et al., 1997). We used a total of 57 highly polymorphic microsatellite markers, which are the most common tools

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applied in the LOH analysis (Dib et al., 1996). The high degree of polymorphism guarantees that the maternal and paternal alleles can be distinguished, i.e. the marker is informative. We have developed three hypotheses in our search for putative tumour suppressor gene regions. First, we analysed the frequency of LOH in different chromosomes for locating putative loci of tumour suppressor genes in the conventional ways. Second, the degree of heterozygosity at the microsatellite markers used was studied because inactivation of a tumour suppressor gene can be detected as LOH even in the tumour-adjacent normal breast tissue (Chen et al., 1999; Deng et al., 1996; Försti et al., 2001). In LOH analysis the heterozygous loci in these regions would be converted to homozygous ones lowering the observed frequency of heterozygosity. Third, concordance of LOH within a twin pair was studied.

Materials and Methods

Sample Collection

Monozygotic twins concordant for breast cancer were identified from the Swedish Twin Registry linked to the Swedish Cancer Registry (Ahlbom et al., 1997). Zygosity was assessed by asking the twins if they as children were as alike as two peas in a pod. When both of the twins gave a positive answer, they were defined as monozygotic (Ahlbom et al., 1997). In our microsatellite analysis an identical allele pattern within each of the twin pairs was observed supporting monozygosity. The cases were diagnosed between 1959 and 1992 in twins born in the period from 1886 through 1958. The number of monozygotic twin pairs concordant to breast cancer was 22. Paraffin-embedded tissue samples were collected from the hospitals of diagnosis. Tumour tissue samples and at least one normal tissue sample per twin pair were available from nine monozygotic twin pairs. These pairs were used for the LOH analysis. Age at diagnosis of these patients ranged from 32 to 76 years (mean 58 years). Six of them were diagnosed at age below 50 years. The mean age of diagnosis of breast cancer in Sweden is close to 62 years. No family history of the twins was available, but one twin (11031) had a missense change in *BRCA2* with a putative pathogenic effect (Försti et al., 2001). None of the twins was a *BRCA1* mutation carrier (Försti et al., 2001).

DNA Isolation

DNA was isolated from the paraffin-embedded tissue samples as described earlier (Försti et al., 1997).

LOH Analysis

LOH analysis was performed mainly as described (Försti et al., 2001). LOH was analysed in the breast cancer samples using 13 microsatellite markers distributed over the whole chromosome 1, 16 markers on the long arm of chromosome 13, 14 markers on chromosome 16, two at the p arm and 12 at the q arm, and 14 markers distributed over the whole chromosome 17 (Figure 1). The primer sequences, heterozygosities and order of the markers as well as genetic distances (in cM) between the microsatellite loci were obtained from the Genome Database (http://www.gdb.org, at Johns Hopkins University) and the Généthon human genetic linkage map (Dib et al., 1996). Primers containing a fluorescent Cy5 dye were obtained from Amersham Pharmacia Biotech and Genset. Unlabelled primers were from Ransom Hill Bioscience, Inc. PCR was performed by using Perking Elmer thermal cycler, model 480. The reaction volume was 5–10µl including 1xPCR buffer (MBI Fermentas), 1–2 mM $MgCl_2$ (MBI Fermentas), 0.11–0.25 mM dNTP, 0.1–1.0 µM primers, and with some primers 5–10% DMSO or glycerol. A "hot start" procedure was used in which 0.25 U Taq polymerase (recombinant, MBI Fermentas) was added at 80°C after an initial denaturation step at 96°C for 2–5 min. PCR was carried out in 27–39 cycles, typically at 94°C for 45s, at 51–65°C for 45s and at 72°C for 45s, with a final elongation step at 72°C for 7–10 min. Amplified products were denaturated at 95°C for 3 min in loading dye (95% formamide containing blue dextran and EDTA, Amersham Pharmacia Biotech). The resulting products were separated on a 6% polyacrylamide-7.2 M urea gel and visualized and analysed with the automated fluorescent ALF Express sequencer (Amersham Pharmacia Biotech). The results were analysed with Pharmacia DNA Fragment Manager 1.2 (Amersham Pharmacia Biotech). For an informative marker, LOH was defined by a decrease of either allele of at least 50%. The LOH analyses were repeated at least twice.

Statistical Analysis

LOH frequency was calculated for each marker, chromosome arm, chromosome and the chromosomes 1, 13, 16 and 17 together by assuming binomial distribution. LOH frequencies at each marker, chromosome arm and chromosome were compared to the mean LOH frequency at chromosomes 1, 13, 16 and 17. The test was considered significant when the 95% confidence interval (95%CI) limits of the observed LOH frequencies on different markers, chromosome arms or chromosomes did not overlap with the 95%CI limits of the observed LOH frequency at chromosomes 1, 13, 16 and 17 together.

The probability of hereditary effect in LOHs between twin pairs was tested by assuming binomial distribution of allelic loss. The expected probability for the twin pairs to lose the same allele by chance was calculated assuming as the null hypothesis H_0: $p(A \cap B \cap C) = p(A) \cdot p(B) \cdot 0.5$, where A is the frequency that one of the twins has LOH, B that the other twin has LOH, C that they have lost the same allele, p is probability. For each chromosome, chromosomal arm and microsatellite locus, the number of twin pairs with loss of the same allele was divided by the total number of informative pairs. The expected numbers were derived from LOH analysis of all the microsatellite markers tested in chromosomes 1p, 1q, 13q, 16p, 16q, 17p and 17q. This is a conservative comparison because each marker was included among the expected numbers. The test was considered significant when the observed probability at a locus was outside the 95%CI limits of the expected probability.

The present analysis involves multiple comparisons but we apply no formal correction for these because our approach is conservative as discussed above and because disagreements exist about the need and type of correction, such as the Bonferroni adjustment (Rothman & Greenland,

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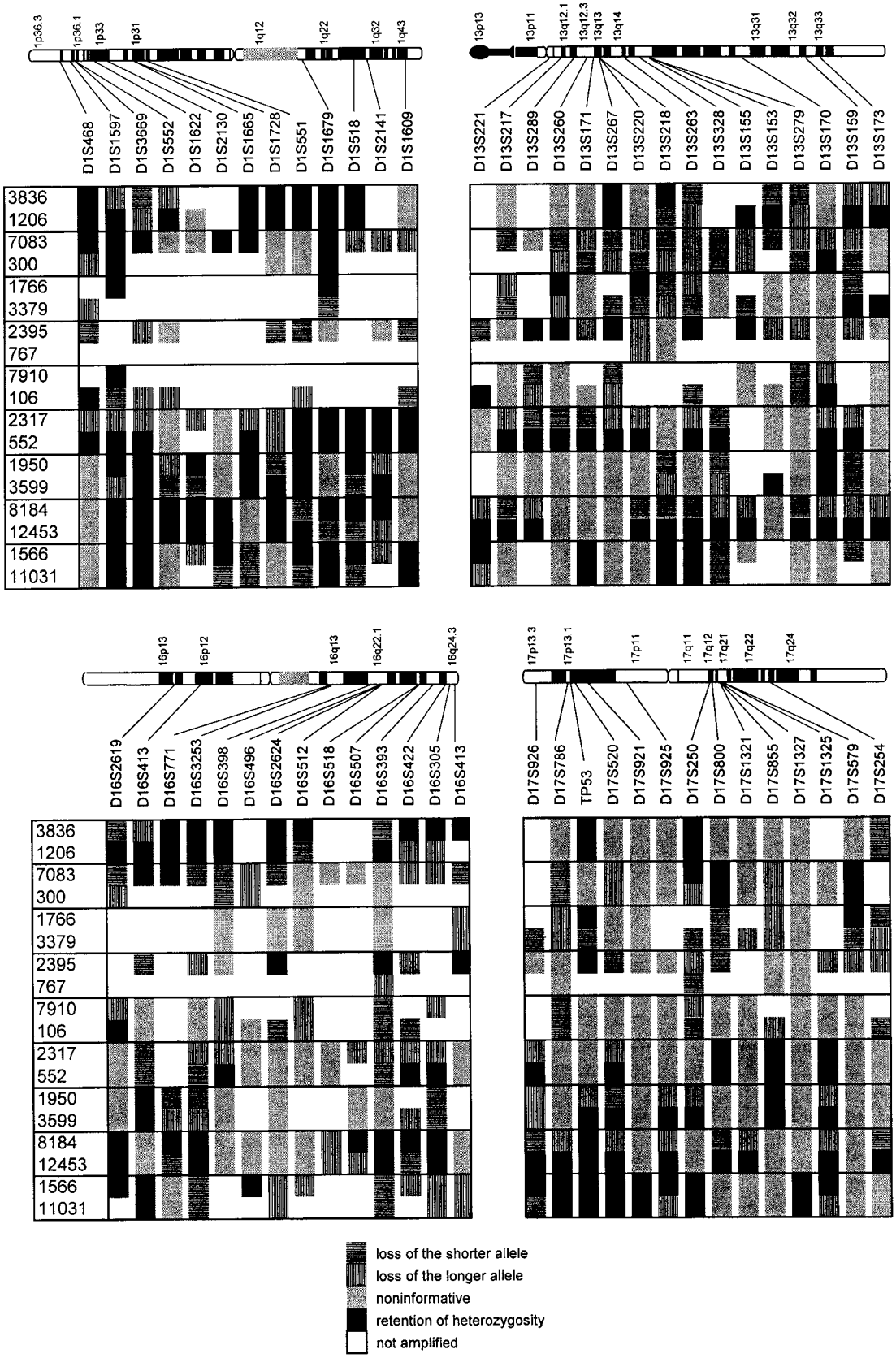

Figure 1

Schematic representation of LOH on chromosomes 1, 13, 16 and 17 in nine monozygotic twin pairs concordant for breast cancer. Patient identification numbers are indicated on the left. Twin pairs are separated from each other by a black line. The markers analysed are shown on the top together with the approximate locations on the respective chromosomes.

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1998). Also we cannot make meaningful power calculations about the twin method because no previous literature exists on the concordance for LOH among monozygotic twins. In addition to our own work, we are only aware of one study, in which genome-wide LOH analysis was carried out in two monozygotic triplet twins (Wistuba et al., 2000).

Results

LOH was analysed on chromosomes 1, 13, 16 and 17 in nine monozygotic twin pairs with breast cancer (Figure 1). Altogether, 37%, 64%, 61% and 51% of the informative samples showed LOH on chromosomes 1, 13, 16 and 17, respectively (Table 1). The mean LOH frequency in the studied chromosomes was 53%. On chromosome arm 1p and the whole chromosome 1, the LOH frequency was significantly lower than the mean LOH frequency on chromosomes 1, 13, 16 and 17. On chromosome arms 13q and 16q, the LOH frequency was significantly higher than the mean LOH frequency. Among individual markers, no increases were noted but one marker, D1S1679, on 1q showed a significantly lower LOH frequency when compared to the overall LOH frequency on chromosomes 1, 13, 16 and 17 (Table 1). On chromosome arm 1q and on marker TP53 on 17p, the LOH frequencies were decreased compared to the mean LOH frequency on chromosomes 1, 13, 16 and 17, but the difference was not statistically significant.

Statistical analysis of the probability for the same allelic loss within a twin pair was carried out by assuming binomial distribution (Table 2). On chromosomes 1, 13, 16 and 17, 32 concordant LOHs with the same allelic loss were observed. They affected 24 markers out of the 57 analysed. The observed frequency of concordant LOH with the same allele lost (0.16) was not statistically different from the expected frequency (0.14). Thus, no general genetic effect of losing the same allele within a twin pair was found. When the four chromosomes were tested separately, the proportion of twin pairs that had lost the same allele was higher than expected on chromosomes 16, 16q and 17p. On chromo-

Table 1

Comparison of the Observed LOH Frequency on Different Chromosomes, Chromosome Arms and Markers to the Observed LOH Frequency on Chromosomes 1, 13, 16 and 17 Combined.

Chromosome	Marker	LOH_{obs}	95%CI
1+13+16+17 combined		0.53	0.50–0.56
1		0.37 ↓	0.28–0.46
1p		0.38 ↓	0.28–0.49
1q		0.34	0.19–0.51
	D1S1679	0.17 ↓	0.02–0.49
13q		0.64 ↑	0.55–0.72
16		0.61	0.51–0.70
16p		0.42	0.20–0.66
16q		0.65 ↑	0.54–0.75
17		0.51	0.41–0.61
17p		0.49	0.34–0.64
	TP53	0.18	0.02–0.52
17q		0.53	0.39–0.66

↑ increased LOH frequency, ↓ decreased LOH frequency, 95%CI limits of the observed LOH frequencies on different chromosomes, chromosome arms and markers do not overlap with the 95%CI limits of the observed LOH frequency of chromosomes 1, 13, 16 and 17 together.

Table 2

Probability (p_{obs}) that the Same Allele is Lost Within a Twin Pair as Compared to the Expected Probability (p_{exp}).

Chromosome	Marker	p_{exp} [a]	CI 95%	p_{obs} [b, c]
1		0.07	0.04–0.10	0.04 (2/51)
1p		0.07	0.04–0.11	0.06 (2/35)
	D1S1665	0.07	0.04–0.11	0.25 (1/4)
	D1S1728	0.07	0.04–0.11	0.25 (1/4)
1q		0.06	0.02–0.12	0 (0/16) ↓
13q		0.20	0.15–0.26	0.12 (7/60) ↓
	D13S220	0.20	0.15–0.26	0.2 (1/5)
	D13S263	0.20	0.15–0.26	0.17 (1/6)
	D13S328	0.20	0.15–0.26	0.4 (2/5) ↑
	D13S155	0.20	0.15–0.26	0.5 (1/2)
	D13S279	0.20	0.15–0.26	0.25 (1/4)
	D13S159	0.20	0.15–0.26	0.17 (1/6)
16		0.19	0.13–0.25	0.31 (14/45) ↑
16p		0.09	0.02–0.21	0.13 (1/8)
	D16S403	0.09	0.02–0.21	0.25 (1/4)
16q		0.21	0.15–0.28	0.35 (13/37) ↑
	D16S3253	0.21	0.15–0.28	0.17 (1/6)
	D16S398	0.21	0.15–0.28	0.50 (2/4) ↑
	D16S496	0.21	0.15–0.28	1.00 (1/1)
	D16S2624	0.21	0.15–0.28	0.33 (1/3)
	D16S512	0.21	0.15–0.28	0.50 (1/2)
	D16S518	0.21	0.15–0.28	1.00 (1/1)
	D16S393	0.21	0.15–0.28	0.33 (2/6) ↑
	D16S305	0.21	0.15–0.28	0.40 (2/5) ↑
	D16S413	0.21	0.15–0.28	1.0 (2/2) ↑
17		0.13	0.09–0.19	0.19 (9/48)
17p		0.12	0.06–0.20	0.22 (5/23) ↑
	D17S926	0.12	0.06–0.20	0.25 (1/4)
	D17S786	0.12	0.06–0.20	0.60 (3/5) ↑
	D17S520	0.12	0.06–0.20	0.20(1/5)
17q		0.15	0.09–0.22	0.16 (4/25)
	D17S800	0.15	0.09–0.22	0.25 (1/4)
	D17S855	0.15	0.09–0.22	0.50 (2/4) ↑
	D17S254	0.15	0.09–0.22	0.33 (1/3)
1+13+16+17		0.14	0.12–0.17	0.16 (32/204)

[a] $p_{exp} = P(A \cap B \cap C) = p(A) \cdot p(B) \cdot 0.5$, where A is the frequency that one of the twins has LOH, B that the other twin has LOH, C is the probability that they have lost the same allele. p_{exp} was calculated for each chromosome and chromosomal arm.

[b] Number of twin pairs with the same LOH / Number of informative twin pairs.

[c] At least two twin pairs with the same LOH were required that the binomial test was considered significant.

↑ Significant difference, p_{obs} exceeds the upper 95 % confidence interval limit of p_{exp}.

↓ Significant difference, p_{obs} lower than the lower 95 % confidence interval limit of p_{exp}.

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Table 3

Comparison of the Observed and Reported Heterozygosity Frequencies at Different Markers, Chromosome Arms and Chromosomes. Binomial Distribution of the Observed Heterozygosity Was Assumed. Reported Heterozygositity Was Compared to the 95%CI Limits of the Observed Heterozygosity.

Chromosome	Marker	$Heterozygosity_{rep}$ [a]	$95\%CI_{obs}$	$Heterozygosity_{obs}$
1		0.75	0.67–0.85	0.77
1p		0.71	0.65–0.87	0.77
1q		0.83	0.60–0.92	0.79
13q		0.79	0.54–0.71	0.63 ↓
	D13S170	0.90	0.22–0.87	0.56 ↓
	D13S173	0.84	0.07–0.70	0.33 ↓
16		0.79	0.57–0.77	0.68 ↓
16p		0.79	0.45–0.92	0.73
16q		0.80	0.57–0.78	0.68 ↓
	D16S398	0.90	0.16–0.84	0.50 ↓
17		0.81	0.41–0.59	0.50 ↓
17p		0.80	0.37–0.66	0.52 ↓
	D17S921	0.74	0.00–0.48	0.11 ↓
17q		0.81	0.37–0.61	0.49 ↓
	D17S1321	0.88	0.03–0.65	0.25 ↓
	D17S1327	0.58	0.00–0.48	0.11 ↓
	D17S579	0.87	0.07–0.70	0.33 ↓
1+13+16+17		0.78	0.60–0.68	0.64 ↓

[a] reported heterozygosity of the different markers are obtained from the Genome Database (http://www.gdb.org) and the Généthon human genetic linkage map (Dib et al., 1996), which were used to calculate the mean heterozygosity of the markers in each chromosome and chromosome arm

↓ observed heterozygosity lower than reported, reported heterozygosity exceeds the upper 95%CI limit of the observed heterozygosity

somes 1q and 13q the observed proportion of the twin pairs with the same LOH was significantly lower than expected.

When the probabilities of losing the same allele within a twin pair at different markers were calculated no difference between the observed and expected probabilities were observed for markers on chromosome 1. On chromosome 13, only one marker (D13S328, between the *BRCA2* and *Rb*-1 locus) showed significantly higher proportion of twin pairs with the same LOH than expected (Table 2). On chromosome 16q more twin pairs with the same LOH were observed at four markers than expected. This explains the higher proportion of twin pairs with the same LOH on both the whole chromosome 16 and the 16q arm. The first marker was D16S398, one of the markers at the *E-cadherin* region. The other three markers were D16S393, D16S305 and D16S413, located at the telomeric end of the q arm. The distance between D16S413 and D16S305 is about 1cM and between D16S305 and D16S393 about 18cM (Dib et al., 1996). On chromosome 17 two markers showed significantly higher proportion of twin pairs with the same LOH than expected (Table 2). The first one was D17S786, distal to the *p53* locus, which explains the higher proportion of twin pairs with the same LOH than expected in the 17p arm. The other marker was D17S855, which is an intragenic *BRCA1* marker. The power of the model is seen in Table 2: even one pair of twins rendered the results formally significant at many markers. We, however, applied a more conservative criterion of at least two twin pairs with the same LOH before calling the findings significant.

Because the proportion of twin pairs, which were heterozygous at a specific marker, seemed to be lower than expected, we tested this notion by assuming binomial distribution (Table 3). The mean observed heterozygosity of the markers on chromosomes 1, 13, 16 and 17 was significantly lower than reported in the literature (64% vs 78%, respectively). On chromosome 1 the reported heterozygosity was within the 95%CI limits of the observed heterozygosity both at the marker, chromosome arm and chromosome level. On chromosome 13q the observed mean heterozygosity was significantly lower than the reported heterozygosity. The twins were more often homozygous than expected at two markers, D13S170 and D13S173, on the telomeric part of the q arm. On chromosome 16 the observed mean heterozygosity of the markers on the q arm was lower than reported, but only one marker, D16S398, was less heterogeneous in the twin pairs than expected. The mean heterozygosities of the markers on chromosome 17, 17p and 17q were significantly lower than reported. On chromosome 17p one marker, D17S921, and on chromosome 17q three markers, D17S1321 and D17S1327, surrounding the *BRCA1* locus, and D17S579, more telomeric, were less heterogeneous in the twin pairs than expected.

Discussion

High LOH frequency at a specific genomic region may indicate a location for a tumour suppressor gene. Hereditary

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effects in the development of breast cancer are seen as concordant loss of the wild type allele at a specific genomic region in the tumours of different members of the same breast cancer family as has been shown in *BRCA1* families (Smith et al., 1992). However, in families with only a few affected individuals, specific regions with concordant loss of the wild type allele are difficult to detect. Monozygotic twins with identical genomes are ideal for the search of putative predisposing tumour suppressor genes by LOH analysis, because the loss of the same allele at a specific locus can reflect the loss of the wild type allele and point out a locus for a tumour suppressor gene. Concordant loss of the same allele at a specific locus in multiple tumours arising in the same individual can also signal heritable risks as has been demonstrated at 17q11-21 in the breast and ovarian tumours of the same patients (Schildkraut et al., 1995). Similarly, the left and right tumours from women with premenopausal bilateral breast cancer had lost the same allele at several markers adjacent or within known breast tumour suppressor genes (Kollias et al., 2000), suggesting a common genetic origin of the tumours.

In our study we have analysed LOH on chromosomes 1, 13, 16 and 17 in monozygotic twins with breast cancer in order to get information on hereditary genetic changes in tumour suppressor genes predisposing to the development of breast tumours. High LOH frequency and low observed heterozygosity compared to reported heterozygosity of the microsatellite markers were used as general indicators for the presence of a tumour suppressor gene. Hereditary effects in the development of breast cancer in twins were suspected when the co-twins had lost the same allele at a specific marker.

By assuming binomial distribution we carried out statistical analysis of the probability for losing the same allele within a twin pair. As comparisons were done in a conservative way within the same chromosome, the high overall rate of LOH on the studied chromosomes (37–64%) may mask moderate increases in LOH rates. On the other hand these kinds of studies always have the problems of chance finding due to multiple comparison. The observed and expected proportions of the twin pairs, which had lost the same allele at markers on chromosomes 1, 13, 16 and 17, were not statistically different (0.16 vs. 0.14, respectively). Thus, no general genetic effect of losing the same allele within a twin pair was found. However, we found two chromosome arms, 16q and 17p, where the proportion of twin pairs with the same LOH was significantly higher than expected (0.35 vs. 0.21, and 0.22 vs. 0.12, respectively), suggesting the presence of predisposing breast tumour suppressor genes.

According to the reasoning given in Introduction most breast tumours in monozygotic twin pairs should be due to inherited cause. The lack of general genetic effect at most chromosomal arms can have a number of explanations. Firstly, we tested only four chromosomes with markers that could not cover even these chromosomes completely. Secondly, LOH analysis detects only loss of function types of defects and is noninformative about gain of function types of defects. Thirdly, as most breast cancers are due to polygenic effects, the different twin pairs may have different complements of susceptibility genes.

Chromosome 16

Both high frequency of LOH (65%), lower degree of heterozygosity than expected (68% vs. 80%) and higher proportion of twin pairs than expected that had lost the same allele (0.35 vs. 0.21, respectively), suggested the presence of at least one tumour suppressor gene on chromosome 16q. At several markers on 16q more twin pairs than expected had lost the same allele. The first one was D16S398, which is located at 16q22.1, near the *E-cadherin* locus (Berx et al., 1998). This marker came also out as one of the markers showing lower heterozygosity than reported, maybe supporting the importance of this region for the tumour development. The other three markers, D16S393, D16S305 and D16S413, where more twin pairs than expected had lost the same allele, are located within 20 cM at the telomere of chromosome 16q. The whole region between D16S393 and D16S413 could have been lost in four twin pairs (3379/1766, 106/7910, 3599/1950 and 11031/1566), making this region as one of the main candidate tumour suppressor gene region in our sample set. This region or part of this region has been implicated in several other LOH studies as well (Chen et al., 1996; Cleton-Jansen et al., 1994; Dorion-Bonnet et al., 1995). Several genes, including *PISSLRE*, *BBC1*, *CMAR*, *FAA*, *GAS1*, *C16orf3*, *DPEP1* and *MC1R* genes, located on this distal part of 16q24.3, have been excluded as candidates for a breast tumour suppressor gene based on either mutation analysis of breast tumour DNA or their expression pattern and known function (Crawford et al., 1999; and references therein). Recently, two other candidate tumour suppressor genes on the more proximal region, but within the region of LOH observed in our study, have been published. A cellular senescence gene, *SEN16*, has been mapped to 16q24.3 (Reddy et al., 1999; Reddy et al., 2000) and a homozygous deletion around the common fragile site FRA16D to 16q23.2 (Paige et al., 2000), providing a basis for identifying putative tumour suppressor genes in these regions.

Chromosome 17

The other main candidate tumour suppressor gene region in our sample set was located at chromosome 17p13, distal to the *p53* locus, as we have shown in our previous study (Försti et al., 2001). *p53* seemed to be an unlikely candidate for a tumour suppressor gene in our sample set, because none of the twin pairs had lost the same allele at the *p53* locus, and only two twins showed LOH at *p53*. In a previous study *p53* germline mutations were shown to be unlikely in premenopausal bilateral breast cancer patients when no concordant LOHs in the two tumours at the *p53* locus were detected (Kollias et al., 2000). The telomeric part of 17p contains a tumour suppressor gene, *HIC-1* (Wales et al., 1995), which has been shown to be hypermethylated in breast tumours (Fujii et al., 1998). Recently, a reduced expression of the human profilin 1 gene, located at 17p13.3, was observed in invasive breast cancer, making it to another putative tumour suppressor gene in this region (Janke et al., 2000). Our results suggest that *HIC-1* or

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another gene distal to the *p53* locus is more important for breast tumour development in our sample set than *p53*.

Chromosomes 17 and 13 contain the two known tumour suppressor genes, *BRCA1* at 17q21 and *BRCA2* at 13q12-13, which predispose to breast cancer (Hall et al., 1990; Miki et al., 1994; Tavtigian et al., 1996; Wooster et al., 1994; Wooster et al., 1995). In our earlier study two twin pairs had lost the same allele at the intragenic *BRCA1* marker D17S855, which was significantly more than expected. However, *BRCA1* mutations could not be analysed from one of them and no *BRCA1* mutations were found in the other twin pair (Försti et al., 2001). The two markers, D17S1321 and D17S1327, flanking the BRCA1 locus, showed significantly lower heterozygosity than reported, giving additional support to the data that another tumour suppressor gene may be located near the *BRCA1* locus causing LOH in sporadic breast cancer (Nagai et al., 1995; Orsetti et al., 1999).

Chromosome 13

The LOH pattern on chromosome 13q was unexpected. The LOH frequency (64%) was one of the highest of the seven chromosome arms studied, and it was significantly higher than the mean LOH frequency of the chromosomes 1, 13, 16 and 17 (53%). Also the observed heterozygosity of the markers on 13q was significantly lower than reported (63% vs 79 %). However, the observed probability of 0.12 for losing the same allele within a twin pair on chromosome 13 was significantly less than expected (0.20). Only one marker on chromosome 13 (D13S328, between the *BRCA2* and *Rb*-1 loci) showed significantly higher proportion of twin pairs with the same LOH than expected. This region was restricted between the *BRCA2* and *Rb*-1 loci, because the first pair 11031/1566, which had lost the same allele at D13S328, had retained heterozygosity at several markers between *BRCA2* and D13S328. The other pair 300/7083 had lost the same allele at D13S328 and at the two markers surrounding it, but the twins had lost a different allele at several markers both around the *BRCA2* and *Rb*-1 loci. None of the twin pairs had lost the same allele at markers D13S260 and D13S171, flanking the *BRCA2* gene, even though we have shown earlier that one sample (11031) contains a missense change with a putative pathogenic effect (Försti et al., 2001). Our results show strong evidence for a tumour suppressor gene to be located on chromosome 13q, the main candidate region being between the *BRCA2* and *Rb-1* loci. This putative tumour suppressor gene is more likely to be important in sporadic breast cancer, as has been suggested earlier (Cleton-Jansen et al., 1995; Eirikdottir et al., 1998), than in hereditary breast cancer.

Chromosome 1

Several distinct regions of LOH both on the short and the long arm of chromosome 1 have been reported both in *in situ* and invasive breast cancer (Hoggard et al., 1995; Munn et al., 1995). $1p_{tel}$ is the most commonly lost region on chromosome 1 in breast cancer (Hoggard et al., 1995; Munn et al., 1995) containing at least one putative tumour suppressor gene, *p73*, a *p53* homologue (Kaghad et al., 1997). In our earlier study by using comparative genomic hybridisation we found one twin pair (12453/8184) and one separate twin (2317), which showed loss of $1p32\text{-}p_{tel}$ (el-Rifai et al., 1999) suggesting the presence of a tumour suppressor gene. In our present LOH study we could not confirm the observed loss. The average LOH frequency on chromosome 1 was only 38%, significantly lower than the mean LOH frequency of chromosomes 1, 13, 16 and 17. Less twin pairs than expected had lost the same allele. Even the observed and reported heterozygosities at different markers were similar. These results show that the possible tumour suppressor genes on chromosome 1 seem not to be involved in the tumour development in our twin pairs.

Conclusions

The monozygotic twin model detects chromosomal regions where non-random loss takes place, i.e. putative inherited susceptibility loci for cancer. According to our present study $16q_{tel}$ and 17p13 are the two main candidate breast tumour suppressor gene regions in Swedish monozygotic twins concordant for breast cancer. Three other minor regions, 16q22.1, 17q21 and 13q14, can also be involved in a subset of the twins. Chromosome 1 does not seem to carry any important tumour suppressor gene in our sample set.

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