Genetic Bases of Estrogen-Induced Tumorigenesis in the Rat: Mapping of Loci Controlling Susceptibility to Mammary Cancer in a Brown Norway × ACI Intercross

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Abstract

Exposure to estrogens is associated with an increased risk of breast cancer. Our laboratory has shown that the ACI rat is uniquely susceptible to 17β-estradiol (E2)–induced mammary cancer. We previously mapped two loci, Emca1 and Emca2 (estrogen-induced mammary cancer), that act independently to determine susceptibility to E2-induced mammary cancer in crosses between the susceptible ACI rat strain and the genetically related, but resistant, Copenhagen (COP) rat strain. In this study, we evaluate susceptibility to E2-induced mammary cancer in a cross between the ACI strain and the unrelated Brown Norway (BN) rat strain. Whereas nearly 100% of the ACI rats developed mammary cancer when treated continuously with E2, BN rats did not develop palpable mammary cancer during the 196-day course of E2 treatment. Susceptibility to E2-induced mammary cancer segregated as a dominant or incompletely dominant trait in a cross between BN females and ACI males. In a population of 251 female (BN × ACI)F2 rats, we observed evidence for a total of five genetic determinants of susceptibility. Two loci, Emca4 and Emca5, were identified when mammary cancer status at sacrifice was evaluated as the phenotype, and three additional loci, Emca6, Emca7, and Emca8, were identified when mammary cancer number was evaluated as the phenotype. A total of three genetic interactions were identified. These data indicate that susceptibility to E2-induced mammary cancer in the BN × ACI cross behaves as a complex trait controlled by at least five loci and multiple gene-gene interactions. (Cancer Res 2006; 66(15): 7793-800)

Introduction

Breast cancer is the most frequently diagnosed nonskin cancer and the second leading cause of cancer-related death in American women. The lifetime risk of a woman in the United States developing breast cancer is approaching one in seven. Numerous studies implicate estrogens in the etiology of breast cancer (1–4).

Known estrogen-related risk factors include early onset of menarche, late onset of menopause, and current or recent use of estrogen replacement or estrogen plus progestin hormone replacement regimens (1, 5–10). Bilateral oophorectomy before menopause markedly reduces breast cancer risk (1, 2). Moreover, the selective estrogen receptor modulator tamoxifen has been shown to reduce by ~50% the incidence of breast cancer in women at high risk of the disease (11, 12). Although it is clear that estrogens are major contributors to breast cancer etiology, the molecular mechanisms through which estrogens induce or promote breast cancer development are not presently defined.

Breast cancer risk is also determined by genetic factors. A small fraction, ~5% to 10%, of breast cancer cases are associated with germ line mutations in one of a small group of genes, including BRCA1, BRCA2, and TP53, for which mutant alleles act in a highly penetrant manner to increase breast cancer risk (13–15). Moreover, genes that encode proteins involved in detoxification of xenobiotics, DNA repair, estrogen metabolism, or signal transduction may function as high prevalence, but low-penetrance, breast cancer susceptibility genes. Although it is clear that only a small fraction of the genetic determinants of breast cancer risk are currently known, the identification of additional bona fide breast cancer susceptibility genes may prove to be difficult due to low penetrance of these genes, gene-gene interactions, or gene-environment interactions. Thus, our understanding of the genetic determinants of breast cancer susceptibility would be facilitated by the use of relevant rodent models that can be evaluated under controlled environmental conditions.

The inbred ACI rat strain serves as a unique resource for defining the mechanisms through which estrogens contribute to breast cancer development and for identifying genes that determine breast cancer risk. Although female ACI rats are highly resistant to spontaneous development of mammary cancer, continuous treatment with physiologic levels of 17β-estradiol (E2) results in rapid development of mammary carcinoma at an incidence approaching 100% (16). The mammary cancers that develop in E2-treated ACI rats are estrogen-dependent adenocarcinomas, a subset of which exhibit invasive behavior (16, 17). Moreover, these mammary cancers frequently exhibit genomic instability, including nonrandom loss of rat chromosome 5 (RNO5),6 which is syntenically related to human chromosomes 1p and 9p, two of the most commonly deleted chromosomal segments in breast cancer (17).
Thus, the mammary cancers induced by E2 in ACI rats share many features in common with breast cancers in humans. The susceptibility of the ACI rat to E2-induced mammary cancer is rat strain specific and genetically conferred (17–20). We have previously mapped to RNO5 and RNO18, two loci, called Emca1 (estrogen-induced mammary cancer) and Emca2, respectively, that determine susceptibility to E2-induced mammary cancer in F2 progeny produced in reciprocal intercrosses between the highly susceptible ACI rat strain and the genetically related, but resistant Copenhagen (COP) rat strain (20). The purpose of this study is to map the genetic determinants of susceptibility to E2-induced mammary cancer in F2 progeny produced in a cross between the ACI rat strain and the resistant Brown Norway (BN) rat strain, which is unrelated genetically to the ACI strain. Data presented herein reveal the existence of five distinct Emca loci that determine susceptibility to E2-induced mammary cancer in this cross. In addition, we define the physical relationships between these Emca loci and loci that control induction of pituitary tumors and uterus infections (21) in these progeny.

Materials and Methods
Care, Treatment, and Phenotypic Characterization of Animals
The Institutional Animal Care and Use Committee of the University of Nebraska Medical Center approved all procedures involving live animals. ACI and BN rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). All procedures relating to care, propagation, and treatment of the experimental animals have been described previously (16–22). Beginning 7 weeks after initiation of E2 treatment, each rat was examined at least weekly for the presence of palpable mammary tumors. The location and size of each palpable tumor were noted at each examination. The rats were killed by decapitation when the largest palpable mammary tumor reached ~2.0 cm in its largest dimension, if necessitated by other treatment-related morbidity, or following 196 ± 3 days of E2 treatment. Mammary tumor number, size, and location were recorded at necropsy. A portion of each tumor was subjected to histologic examination by light microscopy to confirm the existence of mammary carcinoma.

Determination of Genotype and Interval Mapping Analysis
Genotypes were determined at 180 polymorphic simple sequence length polymorphism markers for each of 257 F2 rats. Of these F2 progeny, 230 were euthanized following the full 196-day course of E2 treatment. Twenty-one of the 257 F2 rats exhibited palpable mammary cancer and were euthanized before 196 days due to mammary cancer burden and/or severe uterine infection. The remaining six F2 rats were euthanized before 196 days of treatment due to treatment-related uterine infections or pituitary hyperplasia, but did not exhibit palpable mammary cancer at the time of death. Because the ultimate mammary cancer phenotype could not be determined for these six F2 rats, they were not included in the subsequent genetic analyses. The resulting data for the 251 F2 rats were subjected to interval mapping (IM) and composite IM (CIM) analyses as described by us previously (20, 21, 23). Briefly, genetic maps were constructed and likelihood ratio test statistic (LRS = LOD × 0.30) values, respectively. The incidence of mammary cancer in the population at risk ultimately reached 94% and the number of mammary cancers observed at necropsy averaged 8.8 ± 1.4 per rat in the E2-treated ACI population. By contrast, mammary cancers did not develop in E2-treated BN rats evaluated over the same 196-day course of treatment. Susceptibility to E2-induced mammary cancer was significantly diminished in the F1 and F2 populations, relative to the ACI strain, as evidenced by prolonged latencies to appearance of palpable mammary cancer and reductions in the number of mammary cancers observed at sacrifice (Supplementary Fig. S1; Supplementary Table S1). In the E2-treated F1 population, the mean and median latencies were 167 ± 6 and 168 days, respectively. The incidence of mammary cancer in the population at risk reached 86% and an average of 2.4 ± 0.4 cancers were observed per E2-treated F1 rat. In the treated F2 population, the mean and median latencies were 175 ± 2 and 196 days, respectively. The incidence of mammary cancer in the population at risk reached 58% and an average of 1.9 ± 0.2 mammary cancers were observed at sacrifice in the E2-treated F2 population.

Evaluation of genetic interactions. Potential interaction between the 175 markers resident on the 20 autosomes was evaluated pairwise using MAPMANAGER QTX. Interaction testing was done with the probability of a type 1 error set at ≤0.05. The threshold for significance was obtained by performing 1,000 permutations of the phenotypic data using the interaction model with the level of significance set at P = 0.01 (25, 26).

Statistical Analyses of Data
Latency was defined as the number of days of E2 treatment before the appearance of the first palpable mammary cancer based on weekly examination. Tumor latency was determined using Kaplan-Meier survival estimates and plots. Comparisons of latency among groups were made using the log-rank test. When the overall log-rank test was significant, pairwise log-rank tests were done with P values adjusted using the Bonferroni method. Differences in number of mammary cancers at necropsy and pituitary mass by genotypic class were assessed using the Kruskal-Wallis ANOVA with Dunn’s post hoc tests (GraphPad Prism, version 4.00 for Windows, GraphPad Software, San Diego, CA). P ≤ 0.05 was considered to be indicative of statistical significance.

Results
Susceptibility to E2-induced mammary cancer is genetically determined. Young adult female ACI, BN, (BN × ACI)F1 (F1), and (BN × ACI)F2 (F2) rats were treated with E2 beginning at 9 weeks of age and thereafter were evaluated for mammary cancer development. The ACI and BN rat strains differed dramatically in their susceptibility to E2-induced mammary cancer (Supplementary Fig. S1; Supplementary Table S1). In the ACI population, the first palpable mammary cancer was observed following 80 days of E2 treatment and the mean and median latencies to appearance of the first palpable mammary cancer were 136 ± 6 (SE) and 133 days, respectively. The incidence of mammary cancer in the population at risk ultimately reached 94% and the number of mammary cancers observed at necropsy averaged 8.8 ± 1.4 per rat in the E2-treated ACI population. By contrast, mammary cancers did not develop in E2-treated BN rats evaluated over the same 196-day course of treatment. Susceptibility to E2-induced mammary cancer was significantly diminished in the F1 and F2 populations, relative to the ACI strain, as evidenced by prolonged latencies to appearance of palpable mammary cancer and reductions in the number of mammary cancers observed at sacrifice (Supplementary Fig. S1; Supplementary Table S1). In the E2-treated F1 population, the mean and median latencies were 167 ± 6 and 168 days, respectively. The incidence of mammary cancer in the population at risk reached 86% and an average of 2.4 ± 0.4 cancers were observed per E2-treated F1 rat. In the treated F2 population, the mean and median latencies were 175 ± 2 and 196 days, respectively. The incidence of mammary cancer in the population at risk reached 58% and an average of 1.9 ± 0.2 mammary cancers were observed at sacrifice in the E2-treated F2 population.

Together, these data suggest that susceptibility to E2-induced mammary cancer behaves as a complex genetic trait in this cross originating with BN females and ACI males. It is important to note that mammary cancer did not develop in ovary-intact, sham-treated, ACI, BN, F1, or F2 rats examined over this time frame.

Interval mapping and composite IM analyses reveal Emca loci on RNO3, RNO4, RNO5, RNO6, and RNO7. Phenotype-genotype associations were evaluated for the 251 F2 females for which phenotypic data on mammary cancer susceptibility were available. When mammary cancer status at sacrifice was evaluated as the phenotype, IM analysis revealed regions on RNO7 and RNO3...
where the LRS values exceeded 16.8, the permutation-derived threshold indicative of significant evidence of a QTL (data not shown). CIM analyses of these putative QTL on RNO7 and RNO3 generated statistically significant LRS values for both loci. These QTLs have been designated Emca4 and Emca5, respectively. Emca4 was defined by a peak LRS value near D7Rat19 and a bootstrap-defined confidence interval spanning ~20 cM between D7Rat44 and D7Rat15 (Fig. 1A). Emca5 was defined by a peak LRS value near D3Rat114 and a confidence interval extending over ~75 cM between D3Rat227 and D3Rat210 (Fig. 1B). When the number of mammary cancers observed at necropsy was evaluated as the phenotype, IM analysis identified regions on RNO4 and RNO6 where LRS values exceeded 16.5, the permutation-derived significance threshold for this analysis. Subsequent CIM analyses of these putative QTL generated statistically significant LRS values for each of these QTL. Furthermore, CIM analyses identified a region on RNO5 where the LRS value exceeded the permutation-derived significance threshold. The RNO4 locus has been designated Emca6 and was defined by a peak LRS value proximal to D4Rat103 and a confidence interval spanning ~64 cM from D4Rat14 to D4Rat202 (Fig. 1C). Emca7 mapped to RNO6 and was defined by a peak LRS value proximal to D6Rat22 and an estimated 63 cM confidence interval extending from D6Rat68 to D6Rat81 (Fig. 1D). The RNO5 locus has been designated Emca8, and was defined by a peak LRS value near D5Rat95 and a confidence interval extending over ~50 cM from marker D5Rat134 to D5Rat37 (Fig. 1E). No other region of the rat genome, including RNOX, yielded significant LRS values during IM or CIM analyses. CIM analyses done with the peak markers for each of the Emca loci included in the genetic model as cofactors yielded suggestive evidence for QTL on RNO9, when mammary cancer status at sacrifice was evaluated as the phenotype, and on RNO10 and RNO18, when mammary cancer number at necropsy was evaluated as the phenotype (data not shown).

**Effect of Emca loci on susceptibility to E2-induced mammary cancer.** The ACI alleles for Emca4, Emca5, Emca6, and Emca8 were associated with increased susceptibility to E2-induced mammary cancer (Table 1). Interestingly, the ACI allele for Emca7 was associated with decreased susceptibility. Emca4, Emca5, and Emca8 significantly influenced latency to appearance of mammary cancer and cancer incidence (Fig. 2). For this phenotype, the ACI allele for each of these Emca loci acted in a dominant or an incompletely dominant manner, relative to the BN allele, to decrease latency to mammary cancer appearance. Neither Emca6 nor Emca7 exerted a significant effect on latency to mammary cancer appearance. Figure 3 illustrates the association between genotype at the marker closest to the LRS peak for each of the five Emca loci and the number of mammary cancers observed at necropsy in the E2-treated F2 rats. Each of these Emca loci exerted a statistically significant effect on mammary cancer number. With

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**Figure 1.** Five quantitative trait loci determine susceptibility to E2-induced mammary cancer in F2 progeny from a cross between BN females and ACI males. Genotypes were determined at 180 polymorphic markers for 251 phenotypically defined F2 females from a cross between BN females and ACI males. Tick marks below the horizontal axis, locations of the indicated polymorphic markers. Tick marks above the horizontal axis, 10 cM intervals. The vertical axis represents the LRS values for the correlation between mammary cancer status at sacrifice (A-B) or mammary cancer multiplicity at sacrifice (C-E) and genotype determined by CIM analysis. Solid box, bootstrap-defined confidence interval. Solid and dashed lines, permutation-derived thresholds for significant and highly significant evidence of a locus, respectively.
suggestive evidence of a QTL upon CIM analysis of the F2 population, induced mammary cancer in F2 rats that are homozygous for the BN allele, was unable to suppress susceptibility to E2-induced mammary cancer in F2 rats that are homozygous for the ACI allele at the Emca8-associated marker D5Rat95.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Position marker*</th>
<th>Position (cM)</th>
<th>Peak LRS value $^1$</th>
<th>LOD value$^2$</th>
<th>Percent variance$^1$</th>
<th>Additive effect$^1$</th>
<th>Degree of dominance$^2$</th>
</tr>
</thead>
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<tr>
<td>Mammary tumor status following 196 days of E2 as phenotype</td>
<td>Emca4</td>
<td>D7Rat19</td>
<td>59.0</td>
<td>17.0</td>
<td>3.7</td>
<td>7</td>
<td>0.17</td>
</tr>
<tr>
<td>Mammary tumor number at necropsy as phenotype</td>
<td>Emca6</td>
<td>D4Rat103</td>
<td>49.0</td>
<td>26.6</td>
<td>5.8</td>
<td>10</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Emca7</td>
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<td>4.0</td>
<td>7</td>
<td>−0.94</td>
</tr>
<tr>
<td></td>
<td>Emca8</td>
<td>D8Rat95</td>
<td>80.0</td>
<td>18.7</td>
<td>4.1</td>
<td>7</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*$^1$Simple sequence length polymorphism marker closest to the peak LRS value.
*$^2$Position of peak LRS score in Haldane units relative to first marker on the chromosome.
*$^3$CIM analysis peak LRS value.
*$^4$Additive effect attributed to the QTL by CIM analysis.

Evaluation of physical relationships between Emca determinants of susceptibility to E2-induced mammary cancer and Ept determinants of E2-induced pituitary growth. Two of the five Emca loci identified in this study map to chromosomes that also harbor Ept loci that determine the pituitary growth response to administered E2. Two Emca4 mapped to the same region of RNO7 (Fig. 1A) as Ept7, whereas Emca6 mapped to a region of RNO4 (Fig. 1C) that is ~40 cM proximal to the peak LRS region for Ept5.

Table 1. Actions of Emca loci on E2-induced mammary cancer

To further evaluate the relationship between susceptibility to E2-induced mammary cancer and E2-induced pituitary mass, we examined pituitary mass in the (BN × ACI)F2 population as a function of mammary tumor status following 196 days of E2 treatment (Fig. 5). Pituitary mass in F2 progeny that were free of mammary tumors averaged 31.1 ± 11.9 mg (n = 100), whereas pituitary mass in the mammary tumor–positive rats averaged 40.3 ± 24.1 mg (n = 130). The difference between these two groups of F2 progeny was highly significant (P < 0.001) and was probably a result of the mammary cancer and pituitary mass phenotypes being controlled by linked QTMs: Emca4 and Ept7 on RNO7 and Emca6 and Ept5 on RNO4. The difference in pituitary mass remained significant (P < 0.001) when the pituitary mass data from the 21 F2 rats sacrificed before 196 days were included in the analysis. Thus, we cannot exclude the possibility that the added...
pituitary mass, by producing more prolactin, may have contributed to mammary cancer development.

**Discussion**

Data presented herein indicate that the inbred ACI and BN rat strains differ markedly with respect to susceptibility to E2-induced mammary cancer, and that susceptibility of F2 progeny produced in an intercross originating with BN females and ACI males is determined by at least five Emca loci. ACI alleles for Emca4, Emca5, Emca6, and Emca8, which map to RNO7, RNO3, RNO4, and RNO5, respectively, were associated with increased susceptibility to E2-induced mammary cancer, whereas the ACI allele for Emca7, which maps to RNO6, was associated with reduced susceptibility. Emca1, which was previously mapped to RNO5 in genetic analyses of F2 progeny generated in reciprocal intercrosses between the susceptible ACI strain and the resistant COP strain, overlaps with the Emca8 locus mapped in this study (20). These data suggest that the same gene(s) on RNO5 may determine susceptibility to E2-induced mammary cancer in the crosses between the susceptible ACI strain and the resistant COP or BN strains. Emca2 was previously mapped to RNO18 in F2 progeny generated in an intercross between ACI females and COP males (20). The current study of (BN × ACI)F2 progeny provided suggestive evidence of an Emca locus on RNO18 as well as evidence of a genetic interaction between a marker in the Emca2 region on RNO18 and a marker within Emca7 on RNO6. Together, these data strongly suggest that the Emca2 locus on RNO18 harbors a gene(s) that determines susceptibility to E2-induced mammary cancer. Emca3 was mapped by us to proximal RNO2 in F2 progeny from an intercross originating with ACI females and BN males. No evidence for a QTL residing on RNO2 was generated upon analysis of the (BN × ACI)F2 population evaluated in the current study.

Emca1 and Emca8 both map to the same region of RNO5. We have recently determined that mammary cancers induced by E2 exhibit genomic instability, including frequent and nonrandom loss of RNO5 (17, 20). RNO5 is syntenic to human chromosomes 1p and 9p. Deletion of these chromosomal segments ranks among the most commonly observed cytogenetic aberrations in breast cancers (28–32). We have developed and characterized congenic rat strains that carry the COP allele of Emca1 or the BN allele of Emca8 on the ACI genetic background. Each of these congenic rat strains exhibit significantly reduced susceptibility to E2-induced mammary cancer relative to the parental ACI strain. Together, these data strongly suggest that RNO5 harbors a gene(s) that determines susceptibility to E2-induced mammary cancer whose loss or gain of function may contribute to the genesis of mammary cancers in the ACI rat model of E2-induced mammary carcinogenesis.

Gould and colleagues (33–37) have done genetic studies to define the bases of susceptibility to mammary cancers induced in rats by diethylnitrosamine-2-fluorenylamine (DMBA). In crosses between the susceptible Wistar-Furth (WF) strain and either the resistant COP strain or the resistant Wistar-Kyoto (WKY) strain, these investigators mapped a total of eight QTLs that affect susceptibility to DMBA-induced mammary cancer. One of the QTLs identified in the cross between the WF and WKY rat strains, Mcs5, maps to RNO5 and partially overlaps with Emca8. Unpublished data from 8 M. Tochacek, B.S. Schaffer, and J.D. Shull, unpublished data. 9 B.S. Schaffer, S. Kurz, M. Tochacek, and J.D. Shull, unpublished data.
our laboratory generated during evaluation of several Emca8-derived congenic rat strains, each of which carries the BN allele of a different segment of Emca8 on the ACI background, indicate that an Emca8-associated determinant of susceptibility to E2-induced mammary cancer resides within the region of RNO5 that also contains Mcs5-associated determinants of susceptibility to DMBA-induced mammary cancer (36, 37). However, at least two additional

Figure 3. Effect of Emca loci on mammary cancer multiplicity. Each of the 251 F2 rats was classified according to its genotype at the marker closest to the peak LRS value. Columns, mean number of tumors at sacrifice; bars, SE. Differences in mean tumor number at sacrifice were evaluated using Kruskal-Wallis ANOVA with Dunn's post hoc tests. Numeral 1, significant difference compared with A/A; numeral 2, significant difference compared with B/B; numeral 3, significant difference compared with A/B.

Figure 4. Effect of genetic interactions on susceptibility to E2-induced mammary cancer. Each of the 251 F2 rats was classified according to its genotype at the two markers identified by the interaction function of MAPMANAGER QTX. X axis, genotype information for the designated marker. Genotypes for D18Mit8 (A), D10Rat108 (B), and D7Rat19 (C): ■, A/A; □, A/B; ▲, B/B. A and B, points, mean number of mammary cancers at sacrifice; bars, SE. C, points, percentage of rats that were mammary cancer positive at sacrifice; bars, SE.
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Overlapping with an *Ept* necropsy. Moreover, only one of the pituitary mass and number of mammary cancers observed at mammary cancer. However, no correlation was observed between induced mammary carcinogenesis.

Their susceptibilities to DMBA-induced mammary cancer, strains to E2-induced mammary cancer deviate markedly from (39). Thus, the relative susceptibilities of the ACI, COP, and BN rat strains are both highly resistant to DMBA-induced mammary cancer (33, 38, 39), whereas the BN rat strain is only slightly susceptible to mammary cancer when treated with DMBA (39). Thus, the relative susceptibilities of the ACI, COP, and BN rat strains to E2-induced mammary cancer deviate markedly from their relative susceptibilities to DBMA-induced mammary cancer, suggesting distinct mechanisms for E2-induced and DBMA-induced mammary carcinogenesis.

Two of the *Emca* loci identified in this study, *Emca4* and *Emca6*, map to chromosomes that also harbor *Ept* determinants of pituitary growth and two additional *Emca* loci, *Emca5* and *Emca8*, map to chromosomes that yield suggestive evidence for an *Ept* locus. Consistent with these linkage data and the observed effect of each *Emca* locus on mammary cancer susceptibility and each *Ept* locus on pituitary mass, average pituitary mass in those F2 progeny that did not develop mammary cancer was significantly lower than that observed in those F2 progeny that developed mammary cancer. However, no correlation was observed between pituitary mass and the number of mammary cancers observed at necropsy. Moreover, only one of the *Emca* loci, *Emca4*, directly overlapped with an *Ept* locus, *Ept7*. Therefore, it would seem that susceptibility to E2-induced mammary cancer and induction of pituitary growth are largely controlled by different genetic factors in this study of female (BN × ACI)F2 rats.

We have recently mapped to RNO5 a locus, *Eutr1* (estrogen-induced uterine response 1), that determines susceptibility to E2-induced pyometritis (21). The confidence interval for this locus, which extends from D5Rat116 through D5Rat16, does overlap with the confidence interval for *Emca8*. However, the marker closest to the peak value for *Eutr1*, D5Rat190, is not within the confidence interval for *Emca8* and is ~105 Mb proximal to the peak marker for *Emca8*. Therefore, it is likely that susceptibility to E2-induced mammary cancer and susceptibility to E2-induced pyometritis are controlled by different genes.

The Map Viewer function of the National Center for Biotechnology Information (40) was used to identify known and predicted genes within each of the *Emca* loci mapped in this study. As expected, each of these *Emca* loci contains multiple genes that could potentially act as genetic determinants of susceptibility to E2-induced mammary cancer (Supplementary Tables S2–S6). We are currently using congenic rat strains and substitution mapping approaches to define more precisely the locations of these *Emca* loci so that the genes effecting susceptibility to E2-induced mammary cancer can ultimately be identified.

In summary, we are continuing to use the ACI rat model of E2-induced mammary cancer in genetic studies designed to provide a nonbiased approach toward the identification of genes that determine susceptibility to E2-induced mammary cancer in the rat. We identified in this study five *Emca* loci that determine susceptibility to E2-induced mammary cancer in F2 progeny generated in an intercross between BN females and ACI males. Because of the well-documented role of estrogens in the etiology of breast cancer and the numerous similarities between breast cancers in humans and the mammary cancers that develop in ACI rats upon continuous treatment with physiologic levels of E2, we hypothesize that these *Emca* determinants of susceptibility to E2-induced mammary cancer may similarly function as determinants of breast cancer risk in humans and are working to identify the gene(s) associated with these *Emca* determinants of susceptibility to E2-induced mammary cancer.

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