Genetic Identification of Multiple Loci That Control Breast Cancer Susceptibility in the Rat

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ABSTRACT

We have used a rat model of induced mammary carcinomas in an effort to identify breast cancer susceptibility genes. Using genetic crosses between the carcinoma-resistant Copenhagen (COP) and carcinoma-sensitive Wistar-Furth rats, we have confirmed the identification of the *Mcs1* locus that modulates tumor number. We have now also identified two additional loci, *Mcs2* and *Mcs3*. These three loci map to chromosomes *2*, *7*, and *1*, respectively, and interact additively to suppress mammary carcinoma development in the COP strain. They are responsible for a major portion of the tumor-resistant phenotype of the COP rat. No loss of heterozygosity was observed surrounding the three loci. A fourth COP locus, *Mcs4*, has also been identified on chromosome *8* and acts in contrast to increase the number of carcinomas. These results show that mammary carcinoma susceptibility in the COP rat is a polygenic trait. Interestingly, a polymorphism in the human genomic region homologous to the rat *Mcs4* region is associated with an increased breast cancer risk in African-American women. The isolation of the *Mcs* genes may help elucidate novel mechanisms of carcinogenesis, provide information important for human breast cancer risk estimation, and also provide unique drug discovery targets for breast cancer prevention.

BREAST cancer is a prevalent cancer in the United States population that affects more than 10% of all women. The risk to breast cancer can be modulated by both environmental and genetic factors. Genetic factors include inherited mutant alleles of genes such as p53, BRCA1, and BRCA2. BRCA1 and BRCA2 are found at a low frequency in the U.S. population but are highly penetrant. The penetrance of BRCA1 and BRCA2 was initially estimated to be as high as 85% among heterozygous carriers. This high estimate of penetrance was based on the study of cohorts of very high risk families, many of which were also used to genetically identify these loci. However, not all BRCA1 and BRCA2 carriers are found in such very high risk families. Langston *et al.* (1996) studied a limited-sized population of women who developed breast cancer at an age below 35. Six of 80 women in this cohort carried BRCA1. Only one of these six had a first degree relative with breast/ovarian cancer. Thus, not all BRCA1 carriers are in families with high breast cancer risk. These findings were extended by a recent study by Struewing *et al.* (1997) in which the penetrance of BRCA1 and BRCA2 was estimated in a population of 5318 Ashkenazi Jewish women living in the Washington, DC area. This cohort was analyzed for both breast cancer family history and specific mutations in BRCA1 and BRCA2 associated with this selected population. In contrast to previous studies of high risk families in which a penetrance of 85% was estimated, this community-based study estimated the penetrance of BRCA1 and BRCA2 to be 56%. This finding led to suggestions of caution in interpreting the risk associated with being a carrier of mutant BRCA1 or BRCA2 genes (Healy 1997).

It was hypothesized by the authors of both these studies (Langston *et al.* 1996; Struewing *et al.* 1997) that individuals may carry genes which diminish the consequences of mutant BRCA genes. Such resistance or modifier loci would be very difficult to identify genetically in human populations. This results in part from the difficulty in distinguishing whether families are cancer free because of inherited genes *vs.* other factors or merely because of good fortune. An alternative to the direct study of human populations is to study appropriate rodent models to genetically identify resistance genes. Human homologues of such genes could then be used to directly evaluate their effects on breast cancer risk in human populations.

Mouse and rat models have been used widely for the study of mammary cancer. While each species has its unique merits, mammary cancer in the rat best models human breast cancer. The induced rat carcinoma recapitulates the same histopathologic progression stages to

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malignant breast cancer seen in women. The histopathology of the mouse mammary carcinoma is less similar to the human disease. Rat mammary carcinomas have a responsiveness to hormone treatment similar to that in humans; this is in contrast to the murine cancer in which almost all mammary carcinomas are hormonally refractive (Gould 1995). We thus chose to use a carcinogen-induced [7,12-dimethylbenz-[*a*]anthracene (DMBA)] rat mammary tumor model that is one of the most fully characterized models for both the etiology, prevention, and treatment of human breast cancer.

Rat strains vary greatly in their resistance to carcinogen-induced and spontaneous mammary cancer (Gould *et al.* 1989). The inbred Copenhagen (COP) rat strain is almost completely resistant to mammary carcinogenesis induced by the carcinogen DMBA, as well as to hormone-induced and spontaneous mammary cancers (Dunning and Curtis, 1946, 1952; Isaacs 1986; Gould *et al.* 1989). In contrast to the COP rat, the inbred Wistar-Furth (WF) rat strain is highly susceptible to mammary carcinoma induction by DMBA, with >90% tumor incidence after a single dose of DMBA (Gould *et al.* 1989). Crosses of COP and WF rats were generated to genetically identify genes that modulate susceptibility to mammary cancer.

Using genetic linkage analysis of a (WF × COP)F_1 × WF backcross in which mammary tumors were induced by DMBA, we previously identified a mammary carcinoma susceptibility locus, *Mcs1*, at the proximal (centromeric) end of rat chromosome *2* (Hsu *et al.* 1994). The *Mcs* designation was previously abbreviated mammary carcinoma suppressor because the *Mcs1* COP allele is associated with resistance. Here, we are changing the definition of *Mcs* to mammary carcinoma susceptibility to encompass high susceptibility (for sensitivity alleles) or low susceptibility (for resistance alleles). At the time of the initial identification of *Mcs1*, only a few genetic markers existed to define the rat genome. Only a single minisatellite marker (M13) was linked to the resistance phenotype, and no flanking markers were available because of the scarcity of genetic markers at that time. The M13 marker genetically identified *Mcs1* as being located in a large LOD-1 support interval of 40 cM on chromosome *2*.

Here, we have extended the previous genetic analysis by completing the genome scan in the original backcross panel to search for additional *Mcs* genes, adding more markers to further define and fine map the *Mcs1* region, and generating two additional independent animal crosses to extend/confirm findings from the original backcross. We report here the confirmation of *Mcs1* as a susceptibility locus and the identification of three additional loci that modulate susceptibility to DMBA-induced mammary carcinogenesis: *Mcs2*, *Mcs3*, and *Mcs4* located on rat chromosomes *7*, *1*, and *8*, respectively. The COP allele of *Mcs1* contributes to tumor resistance in a semidominant fashion. The COP alleles of *Mcs2* and *Mcs3* act as dominant resistance loci in heterozygous rats, while the COP *Mcs4* allele acts as a dominant sensitivity locus in heterozygous rats. Results also show that the four loci act additively and account for the great majority of the tumor susceptibility phenotype.

MATERIALS AND METHODS

Animals and phenotyping: COP and WF inbred rats were purchased from Harlan Sprague-Dawley, Inc. (Madison, WI). The mammary tumor phenotype was initially mapped in a (WF × COP)F_1 × WF backcross. For the first backcross (BC1), (WF × COP)F_1 females were mated to WF males and WF females were mated to (WF × COP)F_1 males as described previously (Hsu *et al.* 1994). A second backcross (BC2) was later generated in an identical manner. F_2 intercross animals (F2) were generated by mating F_1 females and males. WF, COP, and F_1 animals were also treated with DMBA for comparison. For each strain or cross, virgin female rats were intubated gastrically with a single dose of DMBA (65 mg/kg in sesame oil) at 55–60 days of age. Mammary tumors were removed at necropsy 18–20 wk after DMBA for WF, 30 wk after DMBA for the F_1, 17–22 wk after DMBA treatment for BC1, and 19 wk after DMBA for the F2 and BC2 crosses. The number of mammary carcinomas (3 × 3 mm diameter or larger) was scored for each rat. DNA was isolated from tail clips taken at weaning or from spleens removed at necropsy, and it was subsequently used for genotype analysis.

Power of crosses to detect quantitative trait loci: The power of each cross to detect loci accounting for certain percentages of the total phenotypic variance in the tumor trait was calculated according to the equation from Lander and Botstein (1989):

$$N = T/\mathrm{ELOD} = T/0.22(\sigma^2_{\mathrm{exp}}/\sigma^2_{\mathrm{res}}),$$

where N is the number of progeny in the cross required so that the LOD score is expected to exceed T, ELOD is the expected LOD score per progeny, $\sigma_{\mathrm{exp}}{}^2$ is the variance explained by the quantitative trait locus (QTL), and $\sigma_{\mathrm{res}}{}^2$ is the residual or environmental variance (which equals the total variance of the cross minus $\sigma_{\mathrm{exp}}{}^2$). This equation was designed to give the number of progeny required for a 50% probability of detection. This number was multiplied by 1.5 to allow for a 90% chance of success.

Source of markers and genotype analysis: Microsatellite markers were obtained from Research Genetics (Huntsville, AL), GenBank, published data, or by collaboration (see acknowledgments). We also generated new microsatellite markers from chromosome-specific (chromosomes *1*, *2*, and *7*), small-insert libraries created in our laboratory (see below).

For genotype analysis, PCR reactions were performed in a 5-μl final volume using 50 ng of genomic DNA template in 96-well plates. Reactions were pipetted using a Biomek 1000 or 2000 automated workstation (Beckman Instruments, Fullerton, CA) and cycled in 96-well thermal cyclers (MJ Research, Watertown, MA). PCR conditions were standard and included 120 nM of each primer and 0.14 μCi of [α-^{32}P]-dATP (3000 Ci/mmol) per reaction. Cycling was as follows: 94° denaturation for 3 min, 25–35 cycles of 94° for 1 min, 55° for 1 min, 72° for 30 sec, and finally 72° for 5 min. PCR products were resolved on polyacrylamide sequencing gels, which were then wrapped in plastic wrap, exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA), and analyzed. When the allele sizes between strains were different enough to be resolved on agarose gels, the PCR was carried out nonradioactively, resolved on 3% MetaPhor agarose (FMC BioProducts,

Rockland, ME), stained with SyBr Green (FMC BioProducts), and scanned on a FluorImager (Molecular Dynamics) for genotype determination.

Generation of additional microsatellite markers using chromosome-specific libraries: To isolate additional markers to fine map QTL regions identified in the genome scan, we generated new microsatellite markers from chromosome-specific, small-insert libraries created in our laboratory (a detailed description of this method will be published elsewhere). Briefly, rat chromosomes were sorted by flow cytometry using methods established previously in our laboratory (Shepel *et al.* 1994) and used to generate small-insert libraries both by a DOP-PCR method (degenerate oligonucleotide–primed PCR) and by an alternative method using restriction endonuclease digestion of the chromosomes. Clones were screened for microsatellite repeats, and oligonucleotide sequences spanning the repeats were synthesized. All novel polymorphic markers from these libraries (designated by the lab code *Uwm*) were synthesized by Research Genetics and are available for purchase from them as Rat MapPairs. Marker *D2Uwm1* (M13) is a minisatellite marker published previously (Hsu *et al.* 1994; Jacob *et al.* 1995) and was mapped by Southern analysis as described by Hsu *et al.* (1994).

Genetic linkage analysis: Genetic maps were generated using the MAPMAKER/EXP 3.0b computer program (Lander *et al.* 1987; Lincoln *et al.* 1993a), and quantitative trait scans were performed using the MAPMAKER/QTL 1.1b computer program (Paterson *et al.* 1988; Lincoln *et al.* 1993b). The initial genome scan in this cross was performed using the parametric scan function in MAPMAKER/QTL program. This parametric analysis assumes a normal distribution of the phenotype; therefore, we used a square root transformation of the tumor number as the phenotype. Such a transformation has been used previously for tumor number phenotypes (Bloom and Falconer 1964; Dietrich *et al.* 1993). This multipoint linkage method allows for detection of loci that may be weak or that may lie between markers, and we chose this for the genome scan to detect any possible linkages.

More recently, a better analysis became available for a phenotype that does not follow a normal distribution. This involves a nonparametric method described by Kruglyak and Lander (1995) and is incorporated into the latest version of MAPMAKER/QTL (version 1.9, 1995). It is based on the Wilcoxon rank sum test (Lehman 1975) with modifications to allow for estimation of linkage between markers. We used the nonparametric QTL analysis with tumor number as the phenotype to compare with the original scan results. This program yields the nonparametric equivalent of the LOD score, the Z_w test statistic, which can be converted to an equivalent LOD_w by the formula $LOD_w = 0.5\ (\log_{10}e)\ (Z_w)^2$ (Kruglyak and Lander 1995). This yielded slightly lower LOD scores than the parametric method.

We also used another program, Qlink, which is based on the statistical methods described by Poole and Drinkwater (1996). Qlink uses a nonparametric method with some modifications of Kruglyak and Lander's methods. One difference is that it does not estimate linkage in the interval between markers, but only at the markers. It is also based on the Wilcoxon rank sum test to obtain the test statistic Z_w for backcross analysis, but it uses a two-sided generalization of the Jonckheere-Terpstra test (Lehman 1975) for intercross data. The test statistics in Qlink are also corrected for tied observations. The latter two methods (*i.e.*, nonparametric using tumor number as the phenotype) are more accurate and conservative. Analyses using all three programs yielded similar results, and we chose to perform all analyses subsequent to the initial genome scan using Qlink.

Joint analysis of crosses: The significance level for linkage as well as determination of the marker having the peak LOD scores for each QTL were determined by a combined analysis of the three crosses using a method described by Fisher (1973). *P* values were obtained from Qlink for each marker in each cross, and the three *P* values at each marker were combined using the formula $(-2\ \Sigma ln\ P)$. This combined value was then converted to a pointwise *P* value as a χ^2 variable with $2n$ degrees of freedom. The combined LOD_w scores for the three crosses were obtained by adding the LOD_w values at each marker from each independent cross. Both the combined *P* and LOD_w values were compared against the genome-wide thresholds listed in the text to determine significance.

Poisson regression model for interaction of loci and gene dosage effects: The two backcrosses and the intercross data were analyzed jointly using Poisson regression models (McCullagh and Nelder 1989) of the following form:

$$\mu_{z,b} = exp(\beta_0 + \gamma_1 b_1 + \gamma_2 b_2 + \beta_1 z_{11} + \alpha_1 z_{12} + \beta_2 z_{21} + \alpha_2 z_{22} + \beta_3 z_{31} + \alpha_3 z_{32} + \beta_4 z_{41} + \alpha_4 z_{42} + z_{m,i} z_{n,i} \xi_{m,i;\ n,i}).$$

For rats with genotype $z_{m,i}$, it was defined that $z_{m,1} = 1$ if the animal had one or two COP alleles at marker *m*, and $z_{m,1} = 0$ otherwise. Also, $z_{m,2} = 1$ if an animal had two COP alleles at marker *m*, and zero otherwise. The tumor count is modeled as a Poisson-distributed random variable with the expected mean $\mu_{z,b}$ (*b* is a function of which backcross the animal came from). The β_0 term represents the baseline tumor rate for intercross animals with no COP alleles, while $b_0 + \gamma_1$ and $b_0 + \gamma_2$ represent the baseline rates in the first and second backcrosses, respectively. The β_m term represents the effect of having one COP allele at marker *m*, and α_m is the added effect of having two COP alleles at that marker. The $\xi_{i,m;i,n}$ term is for interactions between loci for all possible combinations. The model was first fit without the interaction terms, and the Bayes information criterion (BIC; Schwarz 1978) for the model was calculated. Terms were then added if they lowered the BIC and removed if they raised the BIC. By these methods of forward selection and backward elimination, the model with the lowest BIC was selected.

Parameters from the final model were used to calculate a predicted tumor number for each rat. The predicted values were then averaged for each genotypic class to provide the predicted mean tumor numbers, as shown in Table 2.

LOH: LOH was analyzed in mammary tumors of (WF × COP)F_1 rats. Tumors were induced by DMBA, as described above. All rats were palpated for tumors beginning 5 wk after treatment, and rats bearing tumors >1 cm in diameter were killed. Tumors and normal spleen tissue were removed and used for histological and LOH analyses. Tumors were enzymatically digested into ductal fragments as described previously (Chen *et al.* 1996; Haag *et al.* 1996). Briefly, the tumor was finely minced with scissors and transferred into a flask containing collagenase and Dulbecco's modified Eagle's medium. The tumor was digested for ~18 hr with shaking at 37°. The cell mixture was centrifuged to collect the pellet and to remove liquid, fat, and cellular debris. The cell pellet was resuspended in medium, filtered through a 53-μm mesh filter, and washed briefly to remove contaminating stromal components. The ductal fragments remaining on the filter were collected and frozen in liquid nitrogen. By this method, ~95% of the cells isolated were keratin positive.

Based on histopathological analysis, tumors that were adenocarcinomas were used for the LOH study. DNA was isolated from frozen ductal fragments, as well as from normal frozen spleen tissue of the same rat, using standard proteinase K digestion, phenol-chloroform extraction, and precipitation with ethanol. DNA was resuspended in water and used to assay for LOH by the same PCR method described above for genotype analysis. COP and WF alleles were quantitated by

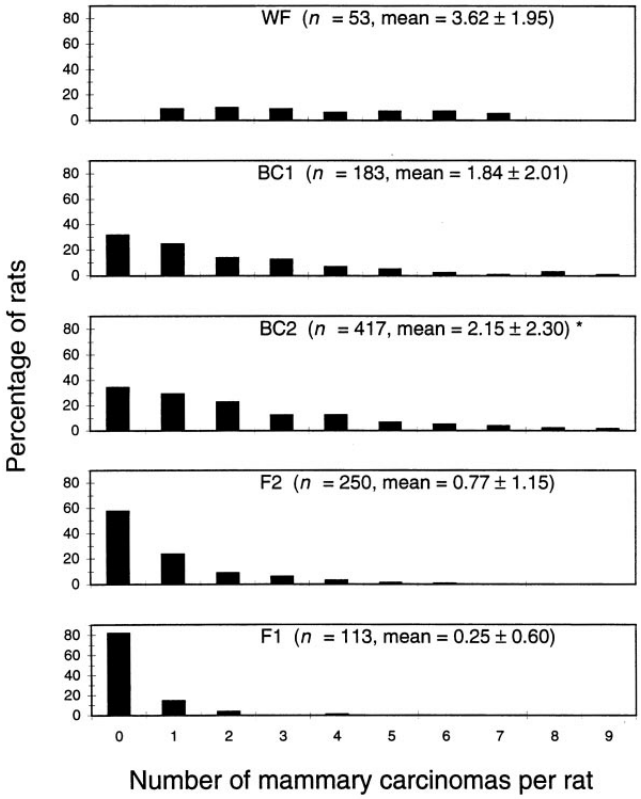

Figure 1.—Distributions of tumor multiplicities for female rats in the WF parent and in four genetic crosses. Rats were treated with DMBA (65 mg/kg in sesame oil) at 55–60 days of age, and the number of mammary carcinomas per rat was determined at necropsy at 17–22 wk of age (see materials and methods). WF, WF parent; BC1, first backcross of (WF × COP)F_1 × WF; BC2, second independent backcross done in the same way as BC1; F2, F_1 × F_1 intercross; F_1, (WF × COP). The number of rats for each group and the mean tumor number (±SD) for the entire population are given in parentheses for each panel. The asterisk for BC2 indicates that there were two animals with 13 and 18 carcinomas that are not shown in the plot. COP rats developed no mammary carcinomas when followed for 25 wk after DMBA (Moore *et al.* 1988).

PhosphorImager scanning, and subsequent analysis was with ImageQuant software (Molecular Dynamics). LOH was defined as a ≥25% difference in the radionuclide incorporation into the PCR products for the COP and WF alleles of the tumor DNA sample relative to the incorporation into allele products for the spleen F_1 control DNA sample.

RESULTS

Inheritance of tumor susceptibility in crosses between COP and WF: Genetic control of tumor multiplicity was examined by looking at the distribution of carcinoma number in parental strains and various genetic crosses. Female rats from an existing (WF × COP) × WF backcross (BC1; Hsu *et al.* 1994), as well as control female WF, COP, and F_1 rats, were treated with DMBA, and the rats were scored for the number of mammary carcinomas at necropsy. COP rats developed no mammary carcinomas (Moore *et al.* 1988) while WF developed an average of 3.6 carcinomas (Figure 1). The distributions of mammary carcinomas in these crosses indicated that the COP strain carries alleles that are at least semidominant in suppressing carcinoma development because the F_1 rats were highly resistant (Figure 1). The backcross (BC1) animals had a carcinoma multiplicity intermediate between the F_1 and WF parent strain, as expected for independent segregation of loci. For the current study, we generated and analyzed two additional crosses: an intercross (F2) and a second backcross (BC2) generated in the same fashion as BC1. In BC2, the mean carcinoma number was again intermediate between the F_1 and the WF parent, as seen for BC1, and the F2 mean was between the backcrosses and the F_1 (Figure 1). Both BC1 and BC2 had similar mean tumor numbers and distributions, and the tumor number ranged from zero to nine in both crosses (except for two rats in BC2 that had 13 and 18 tumors).

Genome scan for linkage in backcross rats: In our first report, as described above, we identified *Mcs1* using 1 minisatellite marker and 113 microsatellite markers in the BC1 panel (this covered 50–75% of the genome; Hsu *et al.* 1994). We have since extended this genome scan by adding markers to the BC1 map and have now tested 349 microsatellites that are polymorphic between COP and WF. Only 10 of the markers were unlinked, resulting in a genome coverage of ~97% (*i.e.*, 339 of 349 markers fell into one of the 21 chromosomal linkage groups, excluding the *Y* chromosome). The majority of the scan is at a minimum resolution of 20 cM, with 16 gaps of 20–37 cM. The average spacing of markers is 5.4 cM, and the total genome size is 2048 cM. This is comparable to the extrapolated female genetic length of 2242 cM estimated by the maps of Jacob *et al.* (1995), and the predicted female length of 2070 cM based on direct chiasmata counts (Robinson 1965). The order of markers and recombination distances on our maps are in general agreement with published maps and the maps of the Rat Genome Project (http://www.genome.wi.mit.edu/rat/public/).

For the genome scan in BC1, 90 rats having the highest and lowest number of carcinomas were chosen for initial genotype analysis to reduce the number of progeny to be genotyped. Selecting these extremes increases the odds of locating a genetic linkage to the phenotype (Lander and Botstein 1989). Any regions having an LOD score of ≥1.0 with this panel were then genotyped in the remaining 93 DNA samples from progeny having an intermediate number of tumors. By this design, we calculated that the initial panel of 90 animals has 50% power to detect a QTL accounting for 3.5% of the total phenotypic variance in the backcross at an LOD of ≥1.0 and accounting for 10% of the variance at an LOD of ≥3.3. Using the full panel of 183 animals and an LOD ≥3.3, there is 50% power to detect loci accounting for 5% of the variance and 90% power to detect those accounting for 8% of the variance.

The initial genome scan in this cross was performed using a parametric scan in the MAPMAKER/QTL 1.1b

program (Paterson *et al.* 1988; Lincoln *et al.* 1993a) with a square root transformation of the carcinoma number as the phenotype. After the initial genome scan, all subsequent analyses were performed using Qlink (see materials and methods). Genome-wide significance thresholds of LOD ≥3.3 and pointwise $P \leq 10^{-4}$ were used as suggested by Lander and Kruglyak (1995) for the dense map case. The thresholds used for suggestive linkage were LOD ≥1.9 and $P \leq 3.4 \times 10^{-3}$.

***Mcs1*:** In our initial study (Hsu *et al.* 1994), we identified the existence of *Mcs1* at the proximal end of rat chromosome *2*. Using tumor number as the phenotype with a parametric scan and analyzing all 183 animals of BC1, the LOD score at minisatellite marker *D2Uwm1* (M13) was 3.8. Because there were no other polymorphic markers available near M13 at that time, the *Mcs1* locus could only be assigned to a large LOD-1 support interval of 40 cM.

To increase the density of the *Mcs1* region, we obtained additional microsatellite markers from various commercial and collaborative sources. In addition to those markers, we generated markers from chromosome-specific libraries made in our laboratory. These markers are highly enriched for chromosome *2*, and five such markers (designated by the code *Uwm*) that are polymorphic between COP and WF have been added to the map in the *Mcs1* region. Using markers from all sources, we now have 12 total markers in the *Mcs1* region for this genetic cross.

Using this relatively dense genetic map, parametric MAPMAKER/QTL analysis with square root of the carcinoma number as the phenotype yielded a peak LOD score of 4.1 at several close markers including *D2Uwm14*. Using the nonparametric Qlink program, a similar LOD_w of 4.4 was observed at marker *D2Rat3* (Table 1). Compared to the initial analysis, the peak location for *Mcs1* shifted only 1–2 cM distal from *D2Uwm1* (M13); however, the additional markers have narrowed the LOD-1 support interval from 40 cM to ~8 cM (Table 1).

Evidence for additional loci *Mcs2*, *Mcs3*, and *Mcs4*: The parametric genome scan in BC1 initially revealed three other loci with LOD scores ≥1.0 on chromosomes *7*, *1*, and *8*, respectively. Additional markers were then added to each chromosomal genetic map to map those regions more densely, and the full panel of 183 animals was tested using all markers. Markers were obtained commercially and via collaborations. We also produced chromosome *7*- and chromosome *1*-specific libraries from flow-sorted chromosomes to generate new markers (*Uwm*).

The resulting maps and scans indicated a significant QTL on chromosome *7* in an interval between markers *D7Mgh15* and *D7Uwm9* (Table 1, peak $LOD_w = 3.38$). We designated the locus in this region as *Mcs2*. A suggestive QTL was observed near markers *D1Mit11* and *D1Wox6* on chromosome *1*. At marker *D1Wox6*, the LOD_w was 2.15 (Table 1), and we tentatively designated this suggestive locus as *Mcs3*. Like *Mcs1*, both of these loci are associated with a decrease in the tumor number in animals carrying COP alleles (relative to animals homozygous for WF alleles), and thus are potential resistance genes.

A region on chromosome *8* indicated a possible QTL near markers *D8Mgh6* and *D8Mgh13*, with a parametric LOD of 1.1 and a Qlink LOD_w of 1.02 (Table 1), which is below the suggestive threshold. However, we pursued the study of this locus because its effect was the opposite of the other three; *i.e.*, the locus was associated with an increase in carcinoma number in rats carrying a COP allele. There was also a small peak with a parametric LOD of 1.1 on chromosome *20* (data not shown), but we have not yet pursued this region. No other chromosomal regions yielded an LOD ≥1. Multiple QTL analyses were also performed in which identified QTLs were fixed (to remove the portion of the variance explained by those loci) and the genome was rescanned; this analysis within the MAPMAKER/QTL program potentially allows identification of additional weaker QTLs. No additional QTLs were found by multiple QTL testing.

Joint analysis of independent crosses for further characterization of potential loci: To confirm significant loci or reach a level of statistical significance of putative loci, two additional independent rat crosses were generated and tested for linkage, as was done for BC1. We first generated an intercross (F2) mapping panel of 250 female rats that were treated with DMBA. The F2 cross allowed for the additional analysis of the effects of two COP alleles at a locus (*i.e.*, homozygous) and for determination of gene interactions. The four loci were tested across the LOD-1 support intervals (from the BC1 analysis) in this F2 panel, and the results are given in Table 1.

A second backcross (BC2) was also generated. This cross contained 417 female animals that were treated with DMBA. DNA samples from all the animals were genotyped for markers in the LOD-1 support intervals of the four QTLs described above. This cross has 90% power to detect loci affecting 3.9% of the variance in the tumor phenotype at an LOD of 3.3 and 50% power to detect loci with 2.9% of the variance. The results in Table 1 indicated that the QTLs on chromosomes *2*, *7*, and *8* are significant in this cross, and the QTL on chromosome *1* is just under the significance threshold ($LOD_w = 3.07$).

For extension studies, data sets can be combined. When the crosses are of the same type, as for two backcrosses, this is best done by pooling the raw data from both crosses, as suggested by Lander and Kruglyak (1995). We thus pooled the raw data from BC1 and BC2 and analyzed it jointly at the four loci. The result with the combined 600 animals yielded significant LOD_w scores for all four loci. The maximum combined LOD_w scores for *Mcs1*, *Mcs2*, *Mcs3*, and the locus on chromosome *8* were 13.1, 7.0, 4.0, and 4.2, respectively. We therefore remove the term tentatively with regard to *Mcs3* and also add the designation of *Mcs4* to the locus on chromosome *8*.

TABLE 1

Linkage of DNA markers to mammary carcinoma induction in three crosses

	Δ position[a]	BC1		F2		BC2		Combined results[b]		
DNA marker	cM	LOD_w	*P*	LOD_w	*P*	LOD_w	*P*	$-2\,\Sigma ln\,P$	Combined *P*	LOD_w
Chromosome *1*										
Lath2	0.7	0.16	0.391	0.18	0.362	0.31	0.232	6.83	3.37E-01	0.65
D1Pas1	8.3	0.02	0.777							
Cype	0.0	0.02	0.735							
Cyp2a1	0.0	0.27	0.261							
D1Uwm1	1.6	0.01	0.849							
D1Mgh5	1.8	0.49	0.132	1.00	0.032	0.19	0.344	13.07	4.20E-02	1.68
Cgm3	11.3	0.10	0.494							
Ton	0.0	1.27	0.016	1.28	0.015	1.38	0.012	25.52	2.74E-04	3.93
Klk1	1.3	0.63	0.088							
D1Mit30	3.4	0.17	0.375							
D1M7Mit69	2.5	0.44	0.153							
D1Mit11	9.3	1.66	5.67E-03	1.48	9.15E-03	1.55	7.52E-03	29.51	4.86E-05	4.69
D1Uwm2	10.4	0.59	0.099	0.90	0.042	1.84	3.59E-03	22.22	1.10E-03	3.33
D1Wox6	3.0	2.15	1.66E-03	0.84	0.049	2.15	1.66E-03	31.64	1.92E-05	5.14
D1Uwm3	0.4	1.27	0.016							
D1Mit2	4.2	1.21	0.018	0.33	0.216	3.07	1.68E-04	28.48	7.62E-05	4.61
D1Mit3	7.3	0.56	0.109	0.54	0.114	1.82	3.81E-03	19.92	2.87E-03	2.92
D1Mit12	2.2	0.51	0.125							
D1Uwm4	6.2	0.54	0.114							
D1Uwm5	12.0	0.49	0.134							
D1Mit13		0.01	0.828							
Chromosome *2*										
D2Mit29	0.6	3.61	4.59E-05	1.73	4.74E-03	9.22	7.31E-11	77.36	1.25E-14	14.56
D2Uwm1	0.5	3.80	2.88E-05							
D2Rat3	1.1	4.40	6.77E-06	2.62	5.07E-04	8.28	6.70E-10	81.23	1.99E-15	15.30
D2Uwm13	0.0	3.83	2.68E-05	2.35	9.96E-04	8.94	1.41E-10	80.24	3.18E-15	15.12
D2Uwm14	0.0	4.31	8.31E-06	1.83	3.65E-03	9.09	9.85E-11	80.70	2.56E-15	15.23
D2Wox2	1.1	4.32	8.09E-06	2.53	6.45E-04	7.53	3.87E-09	76.88	1.57E-14	14.38
D2Rat2	2.7	3.91	2.22E-05	2.87	2.76E-04	7.90	1.61E-09	78.32	7.96E-15	14.68
D2Uwm15	3.5	3.99	1.83E-05	2.11	1.85E-03	4.85	2.31E-06	60.36	3.81E-11	10.95
Ip13dis	2.0	2.56	5.94E-04	1.14	0.022	4.49	5.41E-06	46.75	2.10E-08	8.19
D2Uwm16	6.3	2.53	6.34E-04	1.23	0.018	3.64	4.20E-05	42.92	1.21E-07	7.40
D2Uwm17	3.4	0.77	0.059	0.95	0.037	3.72	3.51E-05	32.77	1.16E-05	5.44
D2Mgh2	10.2	0.21	0.331							
D2Uwm18		0.01	0.793							

(*continued*)

A combined analysis of all three crosses was performed to better define the peak markers using a method described by Fisher (1973), as used by Poole and Drinkwater (1996). Data from the three crosses were combined, and equivalent LOD scores (LOD_w) and *P* values were estimated. Results in Table 1 indicate that there is clearly a mammary carcinoma susceptibility locus, *Mcs1*, near marker *D2Uwm14* on chromosome *2* with a combined LOD_w of 15.23. *Mcs1* is located within a LOD-1 support interval of <6.0 cM. *Mcs2* has a peak combined LOD_w of 7.94 but is currently within a much larger interval of >36 cM. The interval for *Mcs3* is also large (~30 cM), with a peak combined LOD_w of 5.14. In the case of *Mcs4*, the peak LOD_w is 5.11 in a somewhat smaller interval of ~15 cM. Thus, there are four loci affecting the carcinoma phenotype in the COP rat.

Interaction of loci and gene dosage effects: We wanted to assess the relative contributions of each locus to the tumor-resistant phenotype and to identify any gene interactions. Rather than examine the tumor multiplicities for all 81 possible combinations of three genotypes at the four loci, we instead looked at the observed mean tumor numbers in some of the more relevant groups of genotypic combinations using the combined data from all three crosses at the peak markers established in Table 1. The results (Table 2, observed means) indicated that when animals were heterozygous at all four *Mcs* loci, the mean tumor multiplicity (Table 2, group HHHH, mean = 0.95) was similar to that seen in the F_1 hybrid (mean = 0.25 from Figure 1), suggesting that there are likely no additional major loci affecting resistance to tumor development in this rat model. That

TABLE 1

Continued.

	Δ position[a]	BC1		F2		BC2		Combined results[b]		
DNA marker	cM	LOD_w	*P*	LOD_w	*P*	LOD_w	*P*	$-2\,\Sigma ln\,P$	Combined *P*	LOD_w
Chromosome *7*										
D7Uwm1	2.6	2.40	8.79E-04							
D7Mgh11	2.2	2.58	5.69E-04	0.11	0.480	0.96	0.035	23.12	7.59E-04	3.65
D7Mgh9	0.0	2.27	1.21E-03	0.24	0.290	0.55	0.113	20.27	2.48E-03	3.06
D7Mgh15	4.4	2.84	3.02E-04	0.21	0.330	0.21	0.32	20.71	2.07E-03	3.26
D7Uwm7	4.1	3.07	1.68E-04	0.13	0.435	1.06	0.028	26.20	2.04E-04	4.26
D7Mit28	3.1	3.38	7.91E-05	0.16	0.386	3.02	1.92E-04	37.91	1.17E-06	6.56
D7Uwm8	10.9	2.77	3.51E-04							
D7Uwm9	1.8	1.42	0.011							
D7Uwm10	7.7	1.57	7.10E-03	0.61	0.094	3.94	2.06E-05	36.20	2.52E-06	6.12
D7Arb208	1.4	1.13	0.023	0.23	0.304					
D7Uwm11	11.6	1.86	3.38E-03	0.56	0.109	5.52	4.64E-07	44.98	4.72E-08	7.94
D7Mgh10	3.6	1.85	3.53E-03	0.45	0.149	0.95	0.036	21.75	1.34E-03	3.25
D7Uwm12	0.0	1.10	0.024	0.46	0.144					
D7Uwm13	0.0	1.32	0.014							
D7Uwm14	6.7	1.24	0.017							
D7Mit4	0.0	1.28	0.015							
D7Uwm15	1.1	1.34	0.013							
D7Uwm16	3.6	0.76	0.061							
D7Uwm17	11.5	0.75	0.064							
D7Mit11		0.01	0.866							
Chromosome *8*										
D8Mit5		0.65	0.083	0.40	0.175	3.94	2.04E-05	30.06	3.82E-05	4.99
D8Mit4	8.5	0.69	0.074	0.58	0.103	3.56	5.15E-05	29.50	4.89E-05	4.83
D8Mit3	1.7	0.44	0.155	0.63	0.088	4.04	1.60E-05	30.68	2.92E-05	5.11
D8Mgh13	10.4	1.02	0.030	0.08	0.537	1.80	4.01E-03	19.29	3.69E-03	2.90
D8Mit16	0.0	0.69	0.075	0.22	0.318	2.18	1.54E-03	20.42	2.33E-03	3.09
D8Mgh6	1.9	0.98	0.033	0.16	0.392	1.34	0.013	17.38	7.98E-03	2.48
D8Mgh7	1.6	0.52	0.121	0.06	0.596	1.81	3.84E-03	16.38	1.18E-02	2.39
D8Mgh4	9.5	0.57	0.105							
D8Mit1	4.7	0.62	0.092							
D8Mit14	11.3	0.33	0.220							

[a] Δ Position is given as the centimorgan distance (using the Kosambi function) between that marker and the marker below it in the table. Distances were determined in the genome scan using BC1, and they vary slightly in the other two crosses. For each chromosome, only the subset of markers in the regions of the QTLs are shown. The marker orders shown are oriented on the chromosomes with the short arms/centromeres at the top of each list.

[b] Combined results were calculated as described (Fisher 1973). LOD_w was estimated from Qlink Z scores as described (Kruglyak and Lander 1995).

the major loci have been identified was also indicated by the finding that the mean tumor number for animals with no COP alleles at the four loci (*i.e.*, WWWW, mean = 3.47) was nearly identical to that of the WF parent strain (mean = 3.62 from Figure 1). When compared to the WF-like genotype WWWW, the presence of a single copy of the COP allele at *Mcs1*, *Mcs2*, or *Mcs3* tended to reduce the number of carcinomas. When all three *Mcs1*, *Mcs2*, and *Mcs3* loci were heterozygous, the number of carcinomas was reduced by roughly 80% (compare HHHW *vs.* WWWW in Table 2). A single COP allele at *Mcs4* in the absence of the other three alleles appeared to increase the number of carcinomas. As expected, the largest phenotypic difference (~85%) among heterozygous groups was seen between animals that carried one copy of each COP *Mcs1*, *Mcs2*, and *Mcs3* allele with no COP *Mcs4* sensitivity allele (HHHW) and those that carried the sensitivity allele with no *Mcs1*, *Mcs2*, or *Mcs3* alleles (WWWH). Furthermore, although the number of rats in the group was small, it was observed that when rats were homozygous for the COP alleles at all three *Mcs1*, *Mcs2*, and *Mcs3* loci (*i.e.*, six alleles), tumor development was completely suppressed regardless of the presence or absence of the sensitivity allele at *Mcs4* (Table 2, group CCCx, mean = 0). The data shown in Table 2 only indicate trends that needed to be evaluated statistically for significance.

To test the significance of the apparent effects of the *Mcs* alleles and to test for gene interactions, a joint analysis of BC1, BC2, and F2 crosses was performed

TABLE 2

Effect of COP *Mcs* copy number on tumor multiplicity in BC1, BC2, and F2 crosses combined

Genotype[a]				Mean tumor multiplicity		
D2Uwm14 (*Mcs1*)	*D7Uwm11* (*Mcs2*)	*D1Wox6* (*Mcs3*)	*D8Mit3* (*Mcs4*)	Predicted[b]	Observed	No. of rats
W	W	W	W	3.37	3.47	30
H	W	W	W	1.83	1.61	28
W	H	W	W	1.99	1.96	28
W	W	H	W	2.17	2.00	24
W	W	W	H	4.54	4.74	38
H	H	H	W	0.72	0.69	39
H	H	H	H	0.89	0.95	42
C	x	x	x	0.31	0.32	62
x	C	x	x	0.63	0.49	65
x	x	C	x	0.71	0.56	55
x	x	x	C	0.82	0.98	64
C	C	C	x	0.25	0.00	4

Results are for the combined data from BC1, BC2, and F2 rats treated with DMBA (65 mg/kg body wt) and followed for 17–22 wk; the number of mammary carcinomas were counted at necropsy.

[a] Genotypes are as follows: W, homozygous for WF alleles; H, heterozygous for COP and WF alleles; C, homozygous for COP alleles; x, any genotype W, H, or C. The results for the C genotypes were derived from the F2 data. Because of a low number of rats in each F2 genotypic group, the COP homozygote groups (C) were analyzed using any genotype x at the other loci.

[b] Predicted means were calculated from the data obtained using the Poisson regression model (see text and Table 3). The number of rats shown is for the observed data, and the numbers are slightly lower in the first four C groups for the predicted data because of a few missing genotypes at the x loci.

using a Poisson regression model (McCullagh and Nelder 1989). In this model, the additive and dominance components for each locus were determined (Table 3). It was found that for *Mcs1* heterozygous rats, the number of tumors was reduced by 47% compared with animals carrying no COP *Mcs1* alleles. There was also an additive effect at *Mcs1* such that with two COP alleles, the tumor number was reduced by an additional 51% over heterozygotes, for a total reduction of 74% in COP homozygotes. For *Mcs2*, *Mcs3*, and *Mcs4*, the model indicated that there was a significant effect on tumor number when carrying a single COP allele at each locus. When compared with animals homozygous for WF alleles, the tumor number for animals carrying a single COP allele was reduced by 40% for *Mcs2* and by 33% for *Mcs3*. In the case of *Mcs4*, a single COP allele resulted in 36% more tumors than in animals without a COP allele. However, there was no significant difference between heterozygous and homozygous COP groups for *Mcs2*, *Mcs3*, and *Mcs4* (*i.e.*, no α_m terms for these three

TABLE 3

Joint analysis of BC1, BC2, F2 rats for segregation of resistance to mammary carcinoma induction

Parameter	*exp* (estimated parameter), (% change)	*exp* (est $\pm$ 2 $\times$ SE)
β_{Mcs1}	0.535, (−47)	(0.469, 0.609)
α_{Mcs1}	0.490, (−51)	(0.287, 0.836)
$\beta_{Mcs1} + \alpha_{Mcs1}$	0.262, (−74)	(0.154, 0.447)
β_{Mcs2}	0.608, (−40)	(0.538, 0.688)
β_{Mcs3}	0.673, (−33)	(0.594, 0.762)
β_{Mcs4}	1.367, (+36)	(1.206, 1.550)

Tumor multiplicity data from BC1, BC2, and F2 rats were analyzed using a Poisson regression model as described in materials and methods. The parameters shown are the only genetic parameters remaining in the final model determined by the selection procedure. The parameters denote the following: β_m, the effect of having one COP allele at locus *m* (*Mcs1*, *Mcs2*, *Mcs3*, or *Mcs4*); α_m, the added effect of having two COP alleles at locus *m*; $\beta_{Mcs1} + \alpha_{Mcs1}$, the combined effect of having two COP alleles at *Mcs1*. The *exp* (estimated parameter) value is the multiplicative effect of having the parameter indicated; the numbers in parentheses indicate the effective percent decrease or increase in expected tumor number resulting from that parameter. The last column represents the confidence interval for the *exp* (estimate).

TABLE 4

LOH in (WF × COP)F_1 mammary carcinomas induced by DMBA

Mcs locus	Markers tested	Incidence of LOH
Mcs1	*D2Mit29*	0/15
	D2Uwm14	0/15
	D2Uwm16	0/20
Mcs2	*D7Uwm7*	0/12
	D7Mit28	0/11
	D7Arb208	0/20
	D7Uwm11	0/19
Mcs3	*D1Mit11*	0/10
	D1Uwm2	0/10
	D1Wox6	0/19
	D1Uwm3	0/18

loci in Table 3). When all three *Mcs1*, *Mcs2*, and *Mcs3* loci were heterozygous, the number of tumors was reduced by 78%. The addition of a single COP *Mcs4* allele increased the number of tumors slightly so that when animals were heterozygous at all four loci, the tumor reduction was only 70% compared with animals carrying no COP *Mcs* alleles. Inclusion of interaction terms between various loci did not improve the fit of the model ($P > 0.05$).

Using the data from the final Poisson regression model, predicted mean tumor numbers were calculated for each genotypic class in Table 2. The observed and predicted mean values were in good agreement ($r^2 = 0.99$).

LOH: Because LOH is a mechanism by which classical tumor suppressor genes lose their function, we tested for LOH in the DNA from DMBA-induced mammary carcinomas of (WF × COP)F_1 rats. To concentrate the epithelial component of the tumor and remove stromal cell contamination, ductal fragments were isolated from the tumors and then used for isolation of DNA. Tumor and control spleen DNA samples that were previously used to survey the genome for LOH (Haag *et al.* 1996) were used to specifically study the genomic regions surrounding *Mcs1*, *Mcs2*, and *Mcs3* for LOH. In contrast to our previous study with these same DNA samples using the same methodology, where a common LOH on chromosome *1* was delineated, no LOH was found in the regions of *Mcs1*, *Mcs2*, and *Mcs3* (Table 4). These data indicated that loss of the *Mcs* loci does not appear to accompany carcinoma formation in heterozygous rats.

DISCUSSION

We have identified four loci, *Mcs1*, *Mcs2*, *Mcs3*, and *Mcs4*, on rat chromosomes *2*, *7*, *1*, and *8*, respectively, that have significant effects on the induction of mammary carcinomas in the COP rat. Additionally, *Mcs1* is now classified as confirmed because it was significant in two independent studies and reached the *a priori* threshold ($P < 0.01$) for confirmation in a third cross. *Mcs2* was also found to have significant LOD scores in two independent backcrosses and is also classified as confirmed. The current results indicate that resistance to mammary carcinomas in the COP strain is a multilocus trait, including genes that both decrease (*Mcs1*, *Mcs2*, *Mcs3*) and increase (*Mcs4*) susceptibility to induction of carcinomas by DMBA. Taken together, the four loci account for the great majority of the phenotypic difference between the COP and WF strains. The *Mcs* loci act additively to either decrease or increase tumor susceptibility. For *Mcs1*, there is also a gene dosage effect, indicating semidominance. Consequently, it is unknown at this time whether the active allele is from the COP rat or the WF rat. Clearly, the presence of the COP allele of *Mcs1* results in fewer tumors than in its absence, but it is not clear whether the COP allele is active or passive in its action. It may be that it acts indirectly by replacing potentially active WF sensitivity alleles. Furthermore, it is possible that both alleles are active in different ways. For *Mcs2*, *Mcs3*, and *Mcs4*, the Poisson model failed to detect a significant effect of adding a second COP allele, which is compatible with a dominance effect of these three loci. However, it is possible that the additive effect of a second allele was not detected because of the lack of power in the F2 cross.

The *Mcs2* and *Mcs3* loci are currently defined by rather large LOD-1 intervals of 36 and 30 cM, respectively. Given that a large number of animals was analyzed, it is possible that two linked QTLs exist within either or both of these regions. Genotype analysis with additional markers in these regions may resolve this issue. However, the QTL(s) can best be defined by phenotype analysis of congenic rats that have recombinations at various locations within the LOD-1 intervals. Such studies are currently being planned.

The *Mcs* loci do not correspond to the positions in the rat genome of known human breast cancer suppressor genes. p53 and BRCA1 are on rat chromosome *10* (Canzian *et al.* 1996; Chen *et al.* 1996), RB is on rat chromosome *15* (Szpirer *et al.* 1991), and BRCA2 is on chromosome *12* (Yamada *et al.* 1997). To try to identify the chromosomal regions of humans and mice that are homologous to the rat regions containing the *Mcs* loci, we used the comparative gene map databases (Mouse Genome Database; http://www.informatics.jax.org/homology.html). In general, there is a need for gene anchors on the genetic maps to compare maps between species. However, most markers on the rat genetic maps thus far are random sequence markers (primarily microsatellite repeat markers) and do not allow for direct comparisons across species. Additionally, most microsatellite markers are within introns and generally do not amplify unique sequences in other species; thus, they cannot be used to isolate large human or mouse

cosmid or P1 clones, which could be used for fluorescent *in situ* hybridization (FISH) to localize the human or mouse homologous region. Based strictly on the comparative gene maps, we can determine that the gene nearest to *Mcs1* (rat chromosome *2*) that has been mapped in both species is on human chromosome *5q*, *Mcs2* (rat chromosome *7*) has homologies to mouse chromosome *15* and human chromosomes *8*, *22*, and others, and *Mcs3* (rat chromosome *1*) has homologies to mouse chromosomes *7* and *13* and to human chromosomes *5*, *11*, *15q*, and *19*. *Mcs4* (rat chromosome *8*) has homologies to mouse chromosome *9* and human chromosomes *3* and *11*. The only linked candidate rat genes thus far in any of these regions are the cytochrome P450 genes *P-450c* (=*Cyp1a1*; *D8Mgh7*) and *P-450d* (=CYPD45; *D8Mgh13*) in the vicinity of *Mcs4*.

These P450 genes have roles in estrogen and carcinogen metabolism. CYP1A1 in particular is involved in metabolism of environmental xenobiotics such as the polycyclic aromatic hydrocarbons benzo[*a*]pyrene and DMBA. Interestingly, polymorphisms in *Cyp1a1* in humans have been associated with breast cancer risk (odds ratio 9.7) in African-American women (Taioli *et al.* 1995). It is unknown whether this specific (*Msp* I) polymorphism in the African-American population is linked to a functional polymorphism in the CYP1A1 gene or to other genes in this chromosomal region that may control the risk of breast cancer. Our linkage data suggest that the *Mcs4* gene, which is associated with an increased risk of mammary cancer in the COP rat, is slightly proximal to CYP1A1. We thus hypothesize that in African-American women, the CYP1A1 polymorphism may be linked to a gene other than CYP1A1 that could modify susceptibility to breast cancer. If CYP1A1 were involved in controlling mammary carcinogenesis induced by DMBA, one would expect differences in DMBA metabolites or DNA binding among rat strains with differing susceptibilities to cancer development. However, previous studies showed no significant differences in DMBA metabolism or DMBA-DNA binding in mammary epithelial cells of COP and WF rats (Moore *et al.* 1988). Further human and rodent studies are needed to characterize the *Mcs4* region's association with increased risk to breast cancer.

For the *Mcs1* region, there is a new gene marker from this study, *Ip13dis*, located ~9 cM from the peak QTL. We plan to use this gene to help determine the homologous regions in mice or humans. Homology searches for *Ip13dis* in the DNA sequence databases have currently not indicated any homologous genes or expressed sequence tags.

No LOH was detected in the chromosomal regions of *Mcs1*, *Mcs2*, and *Mcs3*. Together with the data discussed above, this finding is compatible with these loci acting as semidominant (*Mcs1*) or dominant genes that contribute to resistance to chemically initiated mammary cancer in the COP rat. Similar findings have been reported for another locus, *Mom1*, which reduces the number of intestinal adenomas caused by the *Apc* gene in Min mice. It was determined that LOH is not observed in the *Mom1* region (Gould *et al.* 1996a). These same investigators also used a *Mom1* congenic mouse strain to determine that *Mom1* affects tumor multiplicity and tumor size in a semidominant fashion (Gould *et al.* 1996b).

Whether the same *Mcs* genes contribute to the resistance of the COP rat to hormonally induced and spontaneous cancer is untested at this time. However, this possibility will be evaluated in rats congenic for each of these genes on a WF background. Such congenics will also be useful to more definitively examine interactions within and between the *Mcs* genes.

While the functions of these *Mcs* genes are unknown, we have previously shown that they collectively act in a cell-autonomous manner. When WF mammary cells were transplanted into (WF × F344)F_1 rats (sensitive to DMBA mammary carcinogenesis) or (WF × COP)F_1 hosts (resistant) to form chimeric rats, the WF transplanted glands were for the most part at similar risk for induced carcinogenesis in both hosts (Zhang *et al.* 1990). In addition, when mixtures of WF and COP mammary cells were grafted into the mammary-free fat pads of (WF × COP)F_1 recipients, the presence of COP cells did not alter the susceptibility of WF cells for induced neoplastic transformation (Zhang *et al.* 1990). These observations suggest that, collectively, the COP *Mcs* alleles act in a cell-autonomous manner in such a way that the presence of these gene products in a mammary cell does not modulate susceptibility in adjacent cells. This latter conclusion assumes a small patch size of each cell strain type in these chimeric glands. Thus, gene product functions that require cell-cell interaction (*e.g.*, paracrine growth factor) are less likely to be associated with the currently identified *Mcs* genes than are gene products whose functions are confined to cells in which the gene product is synthesized (*e.g.*, transcription factors or repair enzymes). However, it is still possible that one or more of the *Mcs* genes may act in a non-cell-autonomous fashion in spite of the above findings. In chimeric glands carrying multiple *Mcs* genes, the possibility exists that the effects of the cell-autonomous alleles predominate and obscure the effects of nonautonomous alleles. Congenic rat strains carrying an individual COP *Mcs* locus will be used in the same transplantation approach to determine whether each *Mcs* locus in isolation acts in a cell-autonomous manner.

Structural and functional information regarding the COP *Mcs* alleles will likely provide insight into understanding the etiology of breast cancer. Our conclusion that multiple genes exist in the rat that can confer resistance to breast cancer supports a possible genetic explanation of limited penetrance of BRCA1 and BRCA2 in many families carrying these sensitivity alleles. Cloning of the *Mcs* resistance genes and defining their human

homologues will provide candidate genes for risk determination in human populations. Functional studies of these resistance genes, together with knowledge of their sequence and structure, will likely provide novel targets for the development of new drugs for the chemoprevention of breast cancer.

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