

Distal Chr 4 Harbors a Genetic Locus (*Gct1*) Fundamental for Spontaneous Ovarian Granulosa Cell Tumorigenesis in a Mouse Model

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Abstract

The spontaneous development of juvenile-onset ovarian granulosa cell tumors in mice of the SWXJ-9 recombinant inbred strain is a model for juvenile-type granulosa cell tumors that appear in very young girls. To expedite gene discovery in this mouse model of childhood cancer, we did a gene mapping study with the SWXJ-9 recombinant inbred strain and the evolutionarily divergent *Mus musculus castaneus* (CAST/Ei) strain as a mapping partner. Our mapping strategy focused on autosomal determinants of susceptibility with a backcross scheme that exploited a paternal, parent-of-origin effect for a X-linked gene (*Gct4*) that strongly supports granulosa cell tumor development. Of 1,968 backcross females examined, we detected 81 granulosa cell tumor-bearing animals and compared their allelic inheritance patterns to non-tumor-bearing siblings in a case-control analysis. The results of our study have confirmed an important locus on mouse chromosome (Chr) 4 (*Gct1*) and have revealed new loci for granulosa cell tumor susceptibility (*Gct7-Gct9*) on Chrs 1, 2, and 13 with susceptibility alleles contributed by the SWXJ-9 progenitor. Two novel gene-gene interactions supportive for granulosa cell tumor development were also observed between loci on Chrs 17 and 18 and loci on Chrs 2 and 10. Our data substantiate the evidence that *Gct1* on Chr 4 is a fundamental oncogene for granulosa cell tumorigenesis in mice and has identified additional interacting autosomal loci that support tumor development. (Cancer Res 2005; 65(4): 1259-64)

Introduction

In infants, girls, and young women, juvenile-type granulosa cell tumors of the ovary represent immediate endocrinological and reproductive complications that require surgical intervention and may pose a life-threatening situation if the cancer is at an advanced stage of growth with local or distant metastases (1). Per annum, the number of cases of juvenile-type granulosa cell tumor is low in North America, thus precluding human genetic linkage studies to identify candidate susceptibility genes for this childhood cancer. To expedite the discovery of granulosa cell tumor susceptibility genes, we are pursuing genetic determinants of susceptibility in the SWXJ-9 recombinant inbred (RI) mouse model that parallels the histological, endocrinological, and developmental timing characteristics of human juvenile-type granulosa cell tumors (2-4).

Juvenile-type granulosa cell tumors were first observed in the SWR/Bm and SWR/J strain females at a very low penetrance (~1-2%), and the SWXJ RI strain set was subsequently derived to confirm the heritable nature of this trait (3). Tumor-susceptible SWR/Bm (SWR) females were mated to tumor-resistant SJL/Bm (SJL) males, and 4 pairs of F₁ offspring were mated to generate 14 F₂ breeder pairs. These F₂ breeder pairs were subsequently inbred for >20 generations to fix large segments of the genome as homozygous SWR or SJL in the SWXJ RI strain set (3, 5, 6). Of the 14 established SWXJ RI lines, SWXJ-9 females show the highest spontaneous granulosa cell tumor incidence, with ~10% of virgin females developing unilateral or bilateral tumors that are macroscopically visible by 6 weeks of age (7). Similar to SWR females, granulosa cell tumor initiation in SWXJ-9 females is restricted to the time of ovarian maturation at puberty, within the window of 3 to 5 weeks of age. Beyond this timeframe, females that have not developed granulosa cell tumors are no longer at risk, have normal reproductive cycles and normal fertility, and develop no other visible pathologic changes in the ovary through 12 months of age. The granulosa cell tumors are endocrinologically active and secrete inhibin and estrogen in the presence of appropriate androgenic substrate, a profile identical to human juvenile-type granulosa cell tumors that leads to symptoms of precocious puberty in young girls or menstrual irregularities in postpubertal women (1, 8-10). The mouse tumors are homogeneously composed of granulosa cells and appear as highly vascularized solid or cystic masses. Between 7 and 12 months of age, granulosa cell tumor-bearing mice may develop multiple metastases with the most commonly seeded organs being renal lymph nodes, liver, and lungs (11). Human juvenile-type granulosa cell tumors also may become malignant, and the diagnosis of advanced-stage disease (III) with tumor spread outside the ovarian capsule confers a poor patient prognosis. Overall, the common biological properties of the mouse and human juvenile-type granulosa cell tumors suggest that similar genetic pathways are involved. Ideally, the identification of genetic determinants for granulosa cell tumor susceptibility in the mouse will provide valuable leads for the discovery of genes involved in juvenile-type granulosa cell tumor etiology in humans.

Two previous mapping studies designed to identify genes associated with granulosa cell tumor development in SWR mice determined that tumor susceptibility is a polygenic trait involving several granulosa cell tumor (*Gct*) susceptibility genes contributed by SWR. Firstly, analysis of the SWXJ RI strain set revealed *Gct1* on Chr 4 as a locus required for spontaneous granulosa cell tumor onset and enhanced tumor initiation by dehydroepiandrosterone (7). Secondly, a (SWR × SJL)_{F₂} intercross mapping study confirmed significant linkage of granulosa cell tumor susceptibility with SWR alleles at *Gct1* on Chr 4 along with *Gct2* on Chr 12, *Gct3* on Chr 15, and *Gct5* on Chr 9 (12).

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In addition to these SWR loci, two X-linked tumor incidence modifier genes (*Gct4* and *Gct6*) have been identified in the SJL strain mapping partner (12, 13). The *Gct4* gene shows a strong parent-of-origin effect: If female mice of an otherwise SWR autosomal background inherit a SJL-derived *Gct4* allele (*Gct4^f*) from their father, there is a dramatically increased risk of developing an ovarian tumor, although the same is not true for maternal inheritance of the *Gct4^f* allele. We have exploited this paternal parent-of-origin effect in a backcross mapping strategy between the granulosa cell tumor-susceptible SWXJ-9 RI strain and the evolutionarily divergent *Mus musculus castaneus* (CAST/Ei) strain. The results of this independent study confirm the essential importance of SWR alleles at *Gct1* for granulosa cell tumorigenesis and have revealed newly identified susceptibility loci on Chrs 1, 2, and 13 along with epistatic loci that support ovarian tumor development.

Materials and Methods

Mice. All mice were produced and housed in our research colony at The Jackson Laboratory (Bar Harbor, ME) under 14:10 hour light/dark cycles. Mice were provided with pasteurized NIH-31 diet (6% fat, Purina Mills International, Brentwood, MO) *ad libitum* and HCl-acidified water (pH 2.8-3.2) *ad libitum* to retard bacterial growth. Animals were weaned at 20 to 23 days of age and housed in groups of 3 to 5 in 329 cm² polycarbonate cages containing sterilized White Pine shavings. All animal procedures were approved by the Animal Care and Use Committee of The Jackson Laboratory.

Backcross Mapping Scheme. Females of the SWXJ-9 strain have the highest spontaneous granulosa cell tumor incidence of any of the 14 SWXJ RI lines and thus were chosen to map susceptibility loci associated with this trait. The SWXJ-9 strain carries the SJL allele at the *Gct4* locus on Chr X that confers strong support for granulosa cell tumorigenesis when paternally inherited (12, 13). For our mapping strategy, we combined the SWXJ-9 characteristic of enhanced granulosa cell tumor penetrance with the rich polymorphic potential of the tumor resistant mapping partner strain CAST/Ei (hereafter, CAST).

Female CAST mice were mated to SWXJ-9 strain males and the F₁ female offspring were backcrossed to SWXJ-9 males to generate N2F1 females. At 8 weeks of age, the N2F1 females were necropsied to assess granulosa cell tumor development by macroscopic examination of the ovaries. At this age, tumors usually present as cystic or solid hemorrhagic masses 5 to 10 mm in diameter and may be bilateral. If a tumor-bearing female was identified, granulosa cell tumors and kidneys were collected from the individual and an unaffected littermate as a control and stored frozen for DNA isolation. Of 1,968 N2F1 females examined, 81 granulosa cell tumor-bearing females were identified (4.1% tumor frequency). No granulosa cell tumors were observed in the 46 F₁ female breeders (mean age at necropsy 43 weeks).

Genotyping. DNA isolated from kidney of N2F1 females was genotyped with a series of 81 simple sequence length polymorphic markers spaced on average 20 cM across the genome. The simple sequence length polymorphic markers used were *D1Mit68*, *D1Mit5*, *D1Mit282*, *D1Mit7*, *D1Mit46*, *D1Mit215*, *D1Mit14*, *D1Mit511*, *D2Mit1*, *D2Mit61*, *D2Mit100*, *D2Mit395*, *D2Mit22*, *D2Mit145*, *D3Mit203*, *D3Mit79*, *D3Mit352*, *D4Mit18*, *D4Mit31*, *D4Mit65*, *D4Mit232*, *D4Mit190*, *D4Mit256*, *D5Mit76*, *D5Mit10*, *D5Mit122*, *D6Mit83*, *D6Mit29*, *D6Mit15*, *D7Mit76*, *D7Mit83*, *D7Mit350*, *D7Mit328*, *D8Mit289*, *D8Mit80*, *D8Mit92*, *D9Mit57*, *D9Mit21*, *D9Mit259*, *D9Mit307*, *D9Mit310*, *D9Mit52*, *D10Mit80*, *D10Mit16*, *D10Mit130*, *D10Mit12*, *D11Mit77*, *D11Mit320*, *D11Mit338*, *D12Mit182*, *D12Mit112*, *D12Mit7*, *D13Mit266*, *D13Mit177*, *D13Mit27*, *D13Mit292*, *D14Mit54*, *D14Mit160*, *D14Mit170*, *D15Mit13*, *D15Mit6*, *D15Mit213*, *D15Mit35*, *D16Mit57*, *D16Mit189*, *D16Mit71*, *D17Mit197*, *D17Mit206*, *D17Mit190*, *D18Mit12*, *D18Mit205*, *D18Mit3*, *D19Mit68*, *D19Mit10*, *D19Mit6*, *DXMit210*, *DXMit96*, *DXMit170*, *DXMit158*, *DXMit231*, and *DXMit35*. Primer pairs were purchased from Integrated DNA Technologies (Coralville, IA) and Qiagen (Valencia, CA).

Simple sequence length polymorphic PCR products were separated by horizontal electrophoresis in 4% MetaPhor agarose slab gels (Cambrex Bio Science, Rockland, ME) and the bands visualized by ethidium bromide staining were photographed for scoring purposes.

Statistical Analyses. Genome-wide scans were carried out for both main effect and interacting quantitative trait loci (QTL) using the method of Sen and Churchill (14). Logarithm of odds ratio (LOD) scores were computed at 2 cM intervals across the genome. Significant loci exceed the genome-wide adjusted $P < 0.05$ level as determined by permutation analysis (15). Simultaneous search genome scans for all pairs of markers were also carried out to detect epistatic interactions (14, 16). Significant QTL by QTL interactions were detected as locus pairs with significant ($P < 0.05$ genome-wide adjusted) joint LOD score and a significant ($P < 0.001$, unadjusted) interaction component. All significant loci and interactions were analyzed jointly in a multiple regression model to obtain unbiased estimates of their effects. Significance levels in the multiple regression may differ from those in the genome scans due to the adjustment for other loci in the model.

Results

The genome wide scan for autosomal loci associated with granulosa cell tumorigenesis in the CAST and SWXJ-9 backcross identified four significant LOD score peaks on four individual chromosomes, Chrs 1, 2, 4, and 13, as shown in Fig. 1. The interval maps for each of these chromosomes are presented in Fig. 2A to D with their assigned locus names. The *Gct1* locus on distal Chr 4 (Fig. 2C) maps to a genetic region previously identified in the F₂ intercross mapping study between SWR and SJL strains (12). The other susceptibility loci are unique to this mapping cross and have been assigned unique locus names: *Gct7* (Chr 1), *Gct8* (Chr 2), and *Gct9* (Chr 13). The interval map for the *Gct7* locus on Chr 1 (Fig. 2A) appears as although two independent susceptibility loci are side by side with peaks at 36 and 48 cM. We explored the evidence for two QTL on Chr 1, but the difference in LOD score (0.5) between a two-locus model and a one-locus model did not achieve significance, however, and our data support only one locus (*Gct7*) for this interval. Table 1 summarizes the chromosomal location, 95% confidence interval, peak DNA markers, and LOD scores for the main effect loci *Gct1*, *Gct7*, *Gct8*, and *Gct9*.

In addition to main effect loci, the analyses for interacting granulosa cell tumor susceptibility loci revealed two significant genetic interactions: one between *Gct8* on Chr 2 (marker *D2Mit145*) and a locus on Chr 10 (marker *D10Mit80*) and a second interaction between a locus on Chr 17 (marker *D17Mit197*) and a locus on Chr 18 (marker *D18Mit12*). The involvement of the Chrs 10, 17, and 18 loci for granulosa cell tumor development was not detected in the overall genome scan and thus represent epistatic loci. A multiple regression model for the cumulative

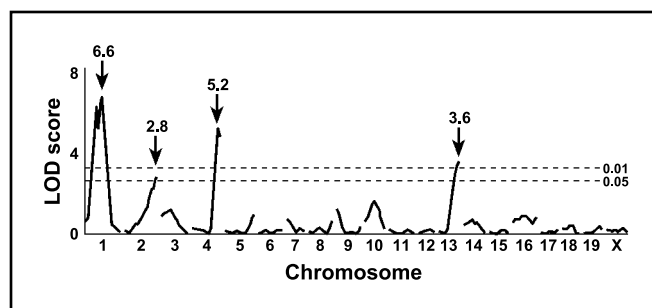


Figure 1. Genome-wide scan for granulosa cell tumor susceptibility loci. Dashed lines, significant ($P = 0.05$) and highly significant ($P = 0.01$) critical levels for LOD scores; arrows, main effect QTL on Chrs 1, 2, 4, and 13.

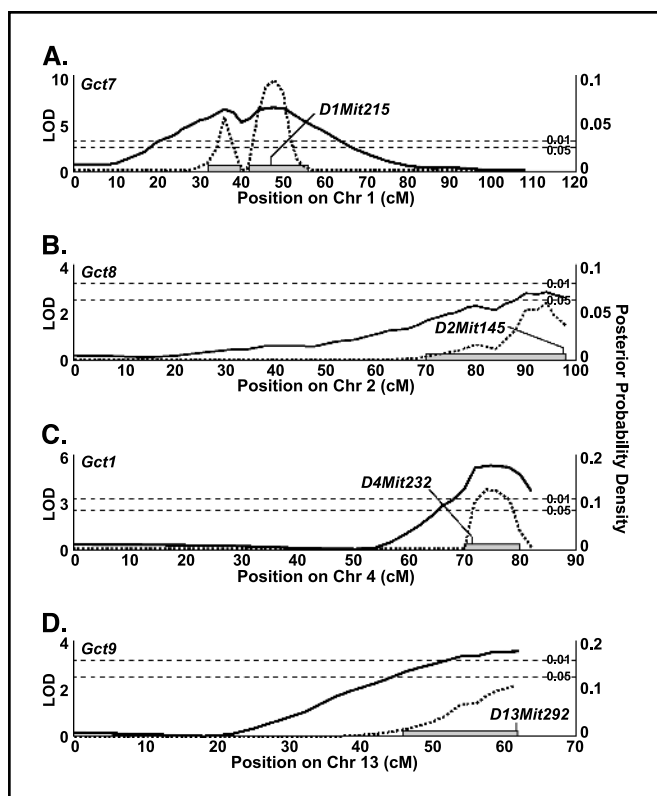


Figure 2. Chromosome interval maps of tumor susceptibility loci with 95% confidence intervals for *Gct7* (A), *Gct8* (B), *Gct1* (C), and *Gct9* (D). Solid lines, region containing the locus; dotted lines, 95% confidence intervals, as determined by posterior probability density. Location of peak DNA markers is identified. Dashed lines, significant ($P = 0.05$) and highly significant ($P = 0.01$) critical levels for LOD scores.

effects of *Gct1*, *Gct7*, *Gct8*, and *Gct9* main effects and the two genetic interactions is presented in Table 2. The four individual loci and two interactions account for 48.2% of the phenotypic variance in this genetic cross.

The genotype ratios for tumor-bearing animals versus unaffected controls are calculated in Table 3 for the peak DNA markers of the *Gct1*, *Gct7*, *Gct8*, and *Gct9* loci. For each marker, the number of tumor-bearing animals with a homozygous SWXJ-9 (*S/S*) genotype was significantly greater than the heterozygous genotype (*S/C*) when compared with the expected ratio of 1:1 for backcross offspring. In contrast, the genotype ratios of unaffected animals were not different from 1:1 between (*S/S*) and (*S/C*). These results show homozygosity for SWXJ-9-derived alleles increases the probability of granulosa cell tumor development. This observation is also graphically depicted in Fig. 3 as allele effect plots for the individual main effect loci and the two genetic interactions. The allele effect plots for *Gct1*, *Gct7*, *Gct8*, and *Gct9* represented by their corresponding peak markers show that alleles derived from the SWXJ-9 strain (*S*) are supportive for granulosa cell tumorigenesis, with increased granulosa cell tumor frequency observed in the homozygous state (*S/S*) compared with the heterozygous state (*S/C*; Fig. 3A-D). In contrast, the interacting genetic loci show unique patterns for enhanced granulosa cell tumor probability. The locus *Gct8* on distal Chr 2 (*D2Mit145*) is further supportive for granulosa cell tumor development if the locus on Chr 10 (*D10Mit80*) is heterozygous for SWXJ-9 and CAST alleles compared with the homozygous

state (Fig. 3E). The second genetic interaction between the Chr 17 locus (*D17Mit197*) and the Chr 18 locus (*D18Mit12*) shows a crosswise interaction for granulosa cell tumor frequency whereby heterozygosity or homozygosity at both loci provides enhanced support for granulosa cell tumor frequency compared with conditions of mixed heterozygosity and homozygosity at either locus (Fig. 3F).

As the SWXJ-9 strain is a recombinant inbred strain composed of SWR and SJL chromosomal regions, we compared the locations of *Gct1*, *Gct7*, *Gct8*, and *Gct9* to the strain distribution map of SWXJ-9 to determine the allelic contributions at these loci (5, 6). Compared with results obtained in the previous mapping study where all autosomal susceptibility alleles were derived from the SWR progenitor, both *Gct7* and *Gct9* reside in regions of SJL genomic material. This indicates that SJL alleles at these autosomal loci can contribute to granulosa cell tumor susceptibility in SWXJ-9 females, although females of the SJL strain do not develop granulosa cell tumors. This assumption awaits confirmation via identification of the *Gct7* and *Gct9* genes and sequencing of both SWR and SJL progenitors. The *Gct8* locus resides in a region of Chr 2 where either SWR or SJL segments could exist; thus, assigning the progenitor source of the active alleles also requires identification of the tumor susceptibility gene and comparison of SWR and SJL variants.

In contrast to *Gct7* to *Gct9*, more confidence can be placed on the allelic contribution of SWR at the *Gct1* locus based on three independent lines of evidence: (a) SWR alleles at the *Gct1* locus are a common feature of the SWXJ RI strains that develop tumors either spontaneously or under the influence of dehydroepiandrosterone, including the SWXJ-9 line; (b) *Gct1*^{SW} was significantly linked with spontaneous tumor development in the (SWR × SJL)_{F2} intercross mapping study; and (c) *Gct1*^{SW}, by virtue of the SWXJ-9 contribution, is again significantly linked with spontaneous granulosa cell tumor development in this backcross mapping study with the CAST strain. These data indicate that *Gct1*^{CAST} and *Gct1*^{SW} alleles are not functionally equivalent and that the *Gct1* locus contains the fundamental gene that confers granulosa cell tumor susceptibility in SWR and SWXJ-9 mice.

Discussion

The objective of our study was to test for genetic determinants in a spontaneous mouse model of juvenile-type granulosa cell tumorigenesis with a mapping cross that possessed greater power for genetic analyses than those previously available. The mapping scheme was designed to identify autosomal loci that support granulosa cell tumorigenesis when phenotypic penetrance of this trait is raised by strong, paternally derived X-linked modifiers of

Table 1. Chromosomal location, peak marker, 95% confidence interval, and LOD score for spontaneous granulosa cell tumor main effect QTL

Name	Chr	Peak (cM)	95% Confidence interval (cM)	Marker	LOD
<i>Gct1</i>	4	74	70-80	<i>D4Mit232</i>	5.2
<i>Gct7</i>	1	48	34-56	<i>D1Mit215</i>	6.6
<i>Gct8</i>	2	96	70-98	<i>D2Mit145</i>	2.8
<i>Gct9</i>	13	60	46-62	<i>D13Mit292</i>	3.6

Table 2. Multiple regression analysis for granulosa cell tumor frequency in (CAST × SWXJ-9)N2F1 progeny

Genetic source	DF	Type III SS	F value	P (f)
D1Mit215	1	3.274	23.748	2.73×10^{-6}
D2Mit145	2	2.374	8.611	0.0003
D4Mit232	1	2.772	20.103	1.44×10^{-5}
D13Mit292	1	1.388	10.066	0.0018
D10Mit80	2	0.640	2.323	0.1014
D17Mit197	2	1.432	5.193	0.0065
D18Mit12	2	1.457	5.283	0.0060
D2Mit145 * D10Mit80	1	0.634	4.596	0.0336
D17Mit197 * D18Mit12	1	1.418	10.282	0.0016
Error	152	20.96		
Total	161	40.50		
Variance explained (%)	48.25			

susceptibility (*Gct4* and *Gct6*; refs. 12, 13). To obtain the highest genetic resolution for localization of tumor susceptibility loci, we chose the evolutionarily divergent CAST mapping partner to contribute a high degree of allelic polymorphism when crossed with the tumor-susceptible, Swiss-derived SWXJ-9 RI strain. The spontaneous granulosa cell tumor frequency observed in N2F1 females derived from this backcross was intermediate (4.1%) compared with that observed in SWXJ-9 females (~10%) or CAST females (0%) and provided 81 granulosa cell tumor-bearing females for linkage analysis. The results of this mapping cross confirmed that granulosa cell tumor susceptibility is a complex trait with multiple individual loci influencing tumor development and provided evidence for significant gene-gene interaction between loci that are not evident in the overall genome scan.

One result of this backcross mapping scheme parallels the previously reported results of the SWXJ RI strain set distribution and the (SWR × SJL)_{F2} intercross—the observation that a strong granulosa cell tumor susceptibility locus (*Gct1*) resides on distal Chr 4 within cytogenetic band E1, with susceptibility alleles contributed by the SWR strain. The evidence for linkage of the *Gct1* locus with granulosa cell tumor susceptibility in three independent mapping crosses using divergent mouse strains satisfies the “burden of proof” principle for *Gct1* as a fundamental gene for juvenile-type granulosa cell tumor development in mice (17). Distal mouse Chr 4 is suspected to harbor tumor suppressor genes that influence the progression of mammary carcinoma and oligoden-

droma in transgenic mouse models based on evidence for loss of heterozygosity with advanced tumor stage (18, 19). Furthermore, the homologous human region to the peak mouse marker *D4Mit232* is Chr 1 at cytogenetic band p36, a region that is frequently deleted in various human cancers and is thus speculated to harbor multiple tumor suppressor genes (20–22). In this mouse model, the *Gct1* locus supports spontaneous ovarian tumor initiation, although it is not yet known if it supplies a classic oncogenic or tumor-suppressive function. The allele effect analysis of granulosa cell tumor-bearing animals shows that heterozygosity for *Gct1*^{SW} was sufficient for tumor development, but homozygosity for *Gct1*^{SW} was more frequently observed in tumor-bearing females. This shows that there is enhanced tumorigenic support in the case of *Gct1*^{SW} homozygosity.

Two attributes of this granulosa cell tumorigenesis model system that should be considered when evaluating candidate genes for the *Gct1* locus are (a) the restricted developmental window with incomplete penetrance of the phenotype in genetically identical female mice and (b) the role of epigenetic regulation as a risk determinant for tumor development. The majority of females genetically susceptible to granulosa cell tumorigenesis who do not develop tumors at puberty are capable of successful reproduction and show no further signs of ovarian pathology through 12 months of age. These facts suggest the active *Gct* susceptibility alleles present a tumorigenic risk at a very restricted period of ovarian development and do not encode mutations with severe functional consequences to reproduction. For instance, females that develop unilateral tumors can undergo tumor resection at 8 weeks of age and exhibit normal fertility with the unaffected ovary following surgery (data not presented). At present, we do not know what stimulates granulosa cell tumor initiation in a fraction of the susceptible population—perhaps subtle differences in the expression of tumor susceptibility genes, environmental factors, or a combination of both. In this respect, the SWXJ-9 mouse model reflects the complexity of the human situation for cancer-associated risk factors (23).

Distal mouse Chr 4 and human Chr 1p36 homologous regions contain genes that can affect the methylation status of the genome, such as methylenetetrahydrofolate reductase, which may affect quantitative differences in gene expression (24, 25). Recognition that epigenetic changes, such as altered methylation status, are equally as important in the process of tumorigenesis, as changes in the DNA sequence of oncogenes or tumor suppressor genes have spurred exploration of global and regional methylation changes in many human tumors (26–28). Furthermore,

Table 3. Genotype of tumor-bearing females and control females at peak markers on Chrs 4, 1, 2, and 13

Peak marker	Genotype ratios of tumor-bearing females		Genotype ratios of normal females		χ^2	P	Relative risk
	S/S	S/C	S/S	S/C			
D4Mit232	71	10	44	37	21.85	<0.001	5.97
D1Mit215	72	9	41	40	28.11	<0.001	7.80
D2Mit145	60	21	39	42	11.45	0.001	3.07
D13Mit292	57	24	32	49	15.58	<1.001	3.63

NOTE: S/S homozygous for SWXJ-9 alleles, S/C heterozygous for SWXJ-9, and CAST alleles.

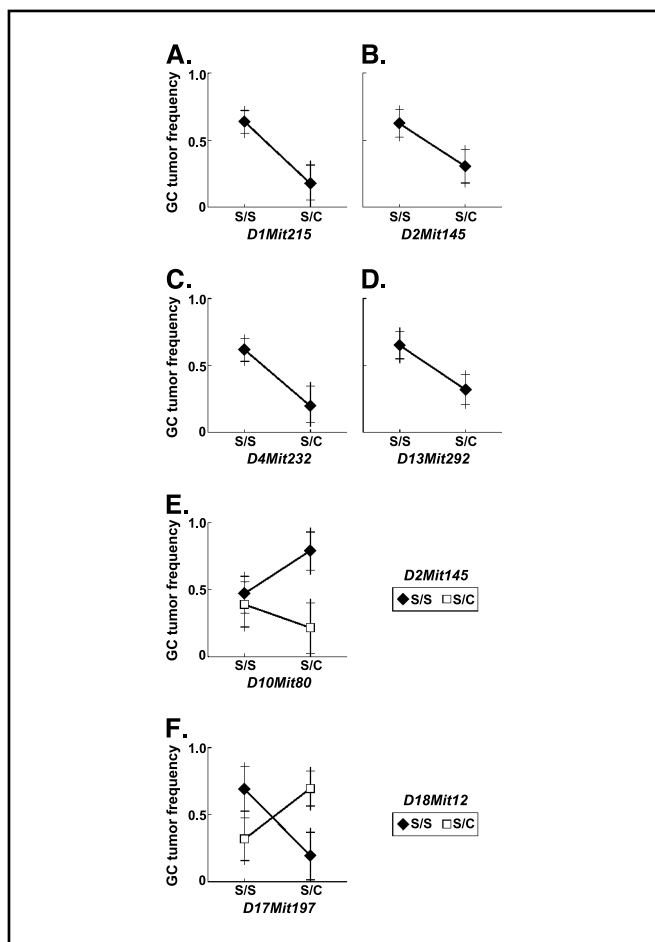


Figure 3. Allele effect plots for *Gct7* (A), *Gct8* (B), *Gct1* (C), and *Gct9* (D), where SWXJ-9 homozygosity is S/S and SWXJ-9/CAST heterozygosity is S/C. E, interaction plot for *Gct8* on Chr 2 and a single locus on Chr 10 (D10Mit80). F, interaction plot for individual loci on Chr 17 (D17Mit197) and Chr 18 (D18Mit12).

epigenetic changes, such as altered methylation patterns, may be of particular relevance for predisposition to childhood tumors (27, 29). The autosomal granulosa cell tumor susceptibility genes active in this mouse model may not be classic oncogenes or tumor suppressor genes but rather modifiers of epigenetic regulation. Fine mapping and positional cloning of the *Gct1* locus will be a priority for testing this hypothesis.

The newly identified *Gct* susceptibility loci observed in this mapping cross (*Gct7* on Chr 1, *Gct8* on Chr 2, and *Gct9* on Chr 13) do not coincide with loci identified for granulosa cell tumor susceptibility in the previous (SWR \times SJL) F_2 intercross, nor are the previous loci (*Gct2* on Chr 12, *Gct3* on Chr 15, and *Gct5* on Chr 9) significant for linkage in this backcross (12). These six loci are thus operative in a strain-dependent manner, although they each have significant influence on the development of granulosa cell tumors. The newly identified *Gct7*, *Gct8*, and *Gct9* loci may reflect important modifier genes that support the elevated level of spontaneous granulosa cell tumorigenesis observed in the SWXJ-9 RI strain. This inference is based on the 5- to 10-fold increased tumor frequency observed in SWXJ-9 females compared with females of the SWR progenitor strain, an increase that cannot solely be attributed to the parent-of-origin effect of X-linked *Gct4* (12). Both *Gct7* and *Gct9* arise from SJL chromosomal segments

of the SWXJ-9 RI strain and could act as modifiers for tumor susceptibility, which have negative activity when the alleles are derived from SWR. An examination of the strain distribution haplotype of another member of the SWXJ RI strain set (SWXJ-8) also suggests that the presence of SJL alleles at *Gct7*, *Gct9*, and perhaps *Gct8* may explain the difference between the 0.4% spontaneous tumor incidence observed in SWXJ-8 and the 10% observed in SWXJ-9 despite their common genotypes at *Gct1* and *Gct4* (5–7). Under this hypothesis, *Gct7*, *Gct8*, and *Gct9* loci would act as strain-dependent, genetic modifiers of granulosa cell tumor susceptibility.

There was evidence for two gene-gene interactions in this backcross mapping study: one was detected between the *Gct8* locus on Chr 2 and a proximal locus on Chr 10. Granulosa cell tumor frequency increased when homozygosity for SWXJ-9 alleles on Chr 2 was combined with heterozygosity at the Chr 10 locus. The second interaction was between two loci on Chrs 17 and 18, which do not seem as significant loci in the overall genome scan. The interaction between the Chrs 17 and 18 loci has an interesting crosswise pattern whereby heterozygosity or homozygosity at either locus has to be matched for increased support of granulosa cell tumorigenesis. Identification of the fundamental genes involved in granulosa cell tumor susceptibility (*Gct1* on Chr 4 and *Gct4* on Chr X) may provide clues to the identity of candidate granulosa cell susceptibility genes at *Gct7*, *Gct8*, and *Gct9* and the interacting loci based on biological associations.

Other mouse models of granulosa cell tumors have been generated with transgene and knockout technologies but differ from the spontaneous SWXJ-9 model, as the tumors can be a mixed population of granulosa cell cells and thecal cells, the phenotypic penetrance is very high, and there is no evidence of a restricted developmental window for granulosa cell tumor susceptibility (30–32). One of these models uses a transgene to overexpress the gonadotropin luteinizing hormone to induce granulosa cell tumors in Swiss-derived CF-1 mice, and granulosa cell tumor susceptibility was still determined to be a strain-dependent and polygenic trait, although no mapping information is currently available for comparison with the *Gct* loci identified in this study (32). Lastly, methods for generating ovarian granulosa cell tumors by carcinogenic exposure or ionizing radiation have been explored in various mouse strains; however, the genetic susceptibility loci remain to be identified (33).

Cytogenetic analyses of human juvenile-type granulosa cell tumors has suggested an association of trisomy Chr 12 and/or monosomy Chr 22 as common features that may be either relevant to or a consequence of tumor development (34–37). The main effect *Gct* loci revealed in this mapping cross do not share regions of homology with either of these human chromosomes. The interacting locus on Chr 10 identified in this mapping cross may have relevance to human granulosa cell tumor cases with trisomy for Chr 12, because human Chr 12 is homologous to a large extent with mouse Chr 10. Given the scarcity of information about specific genes associated with juvenile-type granulosa cell tumorigenesis, we are pursuing genetic determinants in this model of human pediatric cancer based on the strength of common histological and endocrinological characteristics between mouse and human granulosa cell tumors. Because the *Gct1* locus is significantly linked with the granulosa cell tumor phenotype in three independent mapping studies with divergent strains, high resolution mapping efforts are under way for this ovarian granulosa cell tumor locus.

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