Linkage and Microarray Analyses of Susceptibility Genes in ACI/Seg Rats: A Model for Prostate Cancers in the Aged

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Abstract

ACI/Seg (ACI) rats develop prostate cancers spontaneously with aging, similar to humans. Here, to identify genes involved in prostate cancer susceptibility, we did linkage analysis and oligonucleotide microarray analysis. Linkage analysis was done using 118 effective rats, and *prostate cancer susceptibility 1* (*Pcs1*), whose ACI allele dominantly induced prostate cancers, was mapped on chromosome 19 [logarithm of odds (LOD) score of 5.0]. *PC resistance 1* (*Pcr1*), whose ACI allele dominantly and paradoxically suppressed the size of prostate cancers, was mapped on chromosome 2 (LOD score of 5.0). When linkage analysis was done in 51 rats with single or no macroscopic testicular tumors, which had larger prostates and higher testosterone levels than those with bilateral testicular tumors, *Pcs2* and *Pcr2* were mapped on chromosomes 20 and 1, respectively. By oligonucleotide microarray analysis with 8,800 probe sets and confirmation by quantitative reverse transcription-PCR, only two genes within these four loci were found to be differentially expressed >1.8-fold. *Membrane metalloendopeptidase* (*Mme*), known to inhibit androgen-independent growth of prostate cancers, on *Pcr1* was expressed 2.0- to 5.5-fold higher in the ACI prostate, in accordance with its paradoxical effect. *Cdkn1a* on *Pcs2* was expressed 1.5- to 4.5-fold lower in the ACI prostate. Additionally, genes responsible for testicular tumors and unilateral renal agenesis were mapped on chromosomes 11 and 14, respectively. These results showed that prostate cancer susceptibility of ACI rats involves at least four loci, and suggested *Mme* and *Cdkn1a* as candidates for *Pcr1* and *Pcs2*. (Cancer Res 2005; 65(7): 2610-6)

Introduction

Prostate cancer is one of the leading causes of cancer-related death in men in most developed countries, and its incidence is increasing in Japan (1). A variety of genetic and environmental factors are considered involved in the initiation and progression of human prostate cancers (2, 3), and clarification of these factors is urgently required. Genes that are likely to confer dominant susceptibility to prostate cancers have been mapped by linkage analysis of familial prostate cancers at 1q24-25 (4), 1q42-43 (5), 1p36 (6), Xq27-28 (7), 20q13 (8), 17p (9), and 8p22-23 (10). Three putative prostate cancer susceptibility genes, *RNASEL/HPC1* at 1q25 (11), *MSR1* at 8p22 (10), and *ELAC2/HPC2* at 17p11 (9), have been recently identified. In addition, polymorphisms of the *prostate-specific antigen*, isoforms of cytochrome *P*450, *androgen receptor* (12), *vitamin D receptor* (13), and *steroid 5α-reductase 2* (*SRD5A2*; ref. 14) have been proposed to be related to prostate cancer risks. In spite of the efforts and expertise in the field, identification of a responsible gene in each locus and clarification of the interaction among the genes are still facing difficulties due to the complex interactions among these genetic factors in individuals who have been exposed to different levels of various environmental factors.

Experimental animal models are useful because a population of animals can be exposed to the same homogeneous environmental factors and the number of genes involved is limited. As for prostate cancers, a number of rodent models are reported (15). Among these, ACI/Seg (ACI) rats are unique in that they spontaneously develop a high incidence of microscopic cancers of the ventral prostate along with aging (16–18). By 33 months of age, 95% to 100% of the rats develop intra-alveolar dysplasia in the ventral lobe, and 35% to 40% develop invasive carcinomas (17). The high incidence of microscopic cancers and the lower incidence of macroscopic cancers are considered to mimic the natural history of human prostate cancers, where the incidence of microscopic cancers is very high and that of clinical diseases is much lower (19, 20). The ACI model was used to show that a high-fat diet had a promotional effect on the early stage of prostate carcinogenesis (21), that 5α-reductase inhibitor suppresses prostate carcinogenesis (22), and that long-term feeding of a 1% cholesterol diet promotes prostate carcinogenesis, possibly by increasing tissue oxidative stress (23). However, no information is available for the genetic loci or genes responsible for the predisposition of ACI rats to prostate cancers.

In this study, we did linkage and oligonucleotide microarray analyses to identify candidate gene(s) involved in the genetic predisposition of ACI rats to prostate cancers. Linkage analysis was done in F_2 intercross rats produced by crossing ACI rats with F344 rats, which were less susceptible to prostate cancers (24) but more susceptible to spontaneous testicular tumors (25). Oligonucleotide microarray analysis was done considering that if genes differentially expressed in the prostates of ACI and F344 rats are present in the mapped loci, they are good candidates for the responsible genes. Additionally, genes responsible for testicular tumors and unilateral renal agenesis (URA) were mapped.

Materials and Methods

Animals and carcinogenicity test. ACI/SegHgd (ACI) and F344/Jcl (F344) rats were purchased from Harlan (Indianapolis, IN) and CLEA Japan, Inc. (Tokyo, Japan), respectively. Male ACI and female F344 rats were mated to produce F_1 progeny. F_1 rats were intercrossed to produce F_2 intercross

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org).

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Table 1. Results of the carcinogenicity test

	ACI/Seg (n = 12)	F344/Jcl (n = 8)	(F344 × ACI)F_1 (n = 14)	(F344 × ACI)F_2 (n = 118)
Body and organ weights				
Body weight (g)	419.6 ± 65.8 (309.2–506.9)	350.7 ± 29.7 (309.3–391.6)	509.5 ± 67.5 (315.2–583.3)	418.9 ± 77.5 (195.9–652.9)
Prostate weight (g)	1.54 ± 0.83 (0.61–3.00)	0.92 ± 0.19 (0.67–1.26)	1.61 ± 0.75 (0.86–2.91)	1.64 ± 1.58 (0.38–11.75)
Ventral prostate weight (g)	0.32 ± 0.14 (0.13–0.58)	0.27 ± 0.07 (0.19–0.42)	0.36 ± 0.12 (0.22–0.59)	0.35 ± 0.32 (0.07–2.20)
Testes weight (g)	2.81 ± 0.50 (2.04–3.59)	6.62 ± 2.51 (2.48–9.84)	7.73 ± 9.10 (2.57–33.93)	4.20 ± 2.50 (0.67–21.16)
Liver weight (g)	11.3 ± 1.4 (8.5–12.7)	12.7 ± 3.4 (9.6–19.0)	15.0 ± 4.8 (1.5–20.3)	13.2 ± 2.9 (4.7–31.3)
Spleen weight (g)	0.80 ± 0.14 (0.57–1.06)	2.34 ± 1.74 (1.11–5.68)	1.10 ± 0.23 (0.82–1.63)	1.31 ± 0.79 (0.34–5.57)
Any lesions in prostate				
Presence (%)	100	50	100	89
Number	5.3 ± 2.1	1.5 ± 1.9	4.5 ± 1.9	3.8 ± 3.3
Area (mm^2)	1.78 ± 1.22	0.22 ± 0.30	1.03 ± 0.80	1.11 ± 1.50
Area ratio (%)	1.61 ± 1.29	0.25 ± 0.33	0.84 ± 0.59	1.31 ± 1.75
Lesions with a solid structure				
Presence (%)	92	25	93	59
Number	3.0 ± 2.6	0.4 ± 0.7	2.0 ± 1.3	1.3 ± 1.6
Area (mm^2)	1.17 ± 1.25	0.06 ± 0.14	0.40 ± 0.35	0.40 ± 1.07
Area ratio (%)	1.09 ± 1.31	0.07 ± 0.15	0.38 ± 0.37	0.48 ± 1.27
Lesions with ACI-type nuclei				
Presence (%)	100	38	100	81
Number	5.1 ± 2.4	1.3 ± 1.8	3.3 ± 1.4	3.1 ± 3.0
Area (mm^2)	1.73 ± 1.24	0.22 ± 0.30	0.89 ± 0.74	0.90 ± 1.41
Area ratio (%)	1.57 ± 1.31	0.24 ± 0.33	0.73 ± 0.56	1.08 ± 1.68

rats. A total of 21 male ACI rats, 22 male F344 rats, 21 male F_1 rats, and 219 male F_2 intercross rats were fed an Oriental MF (Oriental Yeast Co., Tokyo, Japan) diet and housed three per plastic cage on woodchip bedding in an air-conditioned animal room at a temperature of 21°C to 25°C and a humidity of 40% to 60% with a 12-hour light 12-hour dark cycle. Moribund rats were sacrificed to clarify the cause of death, and the remaining rats were sacrificed at the age of 30 months. The animal experiments followed the Guidelines for the Care and Use of Laboratory Animals of Nagoya City University Medical School and were approved by the Institutional Animal Care and Use Committee.

Histologic and serum analysis. The male sex organs and bladder were resected *en bloc* and were examined for gross abnormalities and fixed in 10% buffered formalin. After fixation, the ventral prostate was removed from the base of the bladder neck and the entire organ weighed. One sagittal slice through the ventral prostate was embedded in paraffin, and three 4-μm-thick sections were stained with H&E. The areas of ventral lobe and lesions were quantitatively measured by an Image Processor for Analytical Pathology (IPAP-WIN, Sumika Technoservice, Takarazuka, Japan). The area of lacuna with secretions was included as the area of the prostate. Hyperplastic and tumorous lesions were classified according to their structural and nuclear atypism as described in Results. The area ratio was calculated as the percentage of the area of a lesion in the total area of the ventral lobe.

Testicular tumors were diagnosed by histologic examination of H&E-stained samples. Leukemia was diagnosed by hypertrophy of the spleen and the liver with infiltration of leukemic cells. Unilateral renal agenesis was diagnosed by the lack of one kidney. All histologic diagnoses were made by experienced pathologists (To.S and S.S.) and a urologist (Y.H.).

Serum testosterone levels are known to be at their peaks between 9:00 am and 1:00 pm (26), and blood samples were collected between 10:00 am and 12:00 noon. Concentrations were measured by RIA at SRL, Inc. (Tokyo, Japan).

Genotyping and linkage analysis. Genotyping was done using 201 markers (157 microsatellite markers and 44 AP-RDA markers; Supplementary Table S1) and genomic DNA extracted from the tails (27–30). Linkage maps were constructed by MAPMAKER/EXP (version 3.0b) software (31), and quantitative trait locus (QTL) analysis was done using MAPMAKER/QTL (version 1.1b) software. The presence of a lesion(s) and absence of any lesions were scored as "1" and "0," respectively, and treated like a QTL. The number of lesions was square root transformed to obtain improved normality. The area and area ratio of lesions were treated as actual values. Association of a quantitative trait with an allele was evaluated by a logarithm of odds (LOD) score. Threshold LOD scores at 2.8 and 4.3 were considered as "suggestive" and "significant" linkages, respectively (32).

Oligonucleotide microarray analysis. Equal amounts of RNA were pooled from the whole prostates of three F344 and three ACI rats at two ages 8 and 48 weeks. Oligonucleotide microarray analysis was done using GeneChip Rat Genome U34A (Affymetrix, Santa Clara, CA) with 8,800 probe sets as in our previous studies (33–35). The signal intensities were normalized so that the average of all the genes on a GeneChip would be equal, and the data were processed using Affymetrix Microarray Suite version 5.0 at the default variable. The annotation was according to RG_U34A Annotations (June 2004; Affymetrix).

Quantitative reverse transcription-PCR. cDNA was synthesized from 2 μg of total RNA with oligo $(dT)_{12-18}$ primer. Real-time PCR analysis was done separately for the three rats in each group using an iCycler iQ detection system (Bio-Rad Laboratories, Hercules, CA) with SYBR Green PCR Core Reagents (Applied Biosystems, Foster City, CA). Primers with the following sequences were used, *Mme* upper 5′- CCTACCGGCCAGAG-TATGCAG -3′ and lower 5′- TATGAGTTCTTGCGGCAATGA -3′, *Cdkn1a* upper 5′- CCTTCTTCTGCTGTGGGTCA -3′ and lower 5′- AAGACACAC-TGAATGAAGGCTA -3′. To quantify the number of molecules of a specific gene in a sample, a standard curve was generated using templates that contained 10^1 to 10^6 copies of the gene and was normalized to that of β-actin.

Results

Incidences and sizes of prostate lesions in inbred and crossed rats. Twelve of 21 ACI, 8 of 22 F344, 14 of 21 F_1, and 118 of 219 F_2 intercross rats survived to 30 months of age,

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premature deaths being caused by renal failure due to URA and leukemia. Hyperplastic and/or tumorous lesions in the ventral prostate were observed in 100%, 50%, 100%, and 89% of ACI, F344, F_1 and F_2 intercross rats, respectively (Table 1). The lesions in ACI and F344 rats had distinct morphologies (Fig. 1). Lesions in ACI rats showed a solid structure, and round, irregular (ACI type) nuclei whereas those in F344 had a papillary or cribriform structure, and angled nuclei with regular sizes. The lesions in ACI rats were much larger (1.8 versus 0.2 mm^2, $P = 0.001$), often associated with inflammatory infiltrates, and occasionally invaded into the adjacent alveoli. In contrast, lesions in F344 rats never developed into macroscopic cancers. Based on the structural and nuclear atypisms and sizes of tumors, ACI ($n = 12$) and F344 ($n = 8$) rats could be distinguished with 95% accuracy in a blinded manner.

Therefore, all the lesions were classified according to their structural and nuclear atypisms (Table 1). A solid structure was present in 92%, 25%, 93%, and 59% of ACI, F344, F_1 and F_2 intercross rats, respectively. ACI-type nuclei were present in 100%, 38%, 100%, and 81%, respectively. In ACI and F_2 intercross rats, 100% and 99%, respectively, of lesions with a solid structure had ACI-type nuclei.

Testicular tumors, unilateral renal agenesis, leukemia, and subcutaneous tumors. Testicular tumors, histologically diagnosed as Leydig cell tumors, were observed in 92%, 100%, 100%, and 92% of ACI, F344, F_1 and F_2 intercross rats, respectively. Bilateral macroscopic testicular tumors were observed in 25%, 88%, 100%, and 57%, respectively. URA was observed in 24%, 0%, 0%, and 11%, respectively, at 30 months. Leukemia was observed in 0%, 25%, 0%, and 9%, respectively, at 30 months. S.c. tumors were observed in 0%, 38%, 0%, and 10%, respectively.

Linkage mapping of a gene that affects the development and the size of prostate cancers. Linkage analysis with the presence of (i) any lesions, (ii) lesions with a solid structure, and (iii) lesions with ACI-type nuclei was done in the 118 F_2 intercross rats, and one QTL was identified in the vicinity of *D19Rat75* (LOD scores of 2.9, 3.0 and 5.0, respectively; Table 2). The same QTL was mapped using the numbers of these three kinds of lesions. When F_2 intercross rats were classified by the genotypes at *D19Rat75*, lesions with ACI-type nuclei were observed in 85% (22 of 26), 92% (60 of 65), and 52% (14 of 27) of rats with the ACI/ACI, ACI/F344, and F344/F344 genotypes, respectively. This locus, *prostate cancer susceptibility 1* (*Pcs1*), therefore conferred ACI-dominant prostate cancer susceptibility (Fig. 2*A*). This region (*D19Rat75-D19Rat46*) corresponded to rat 19q11-12.

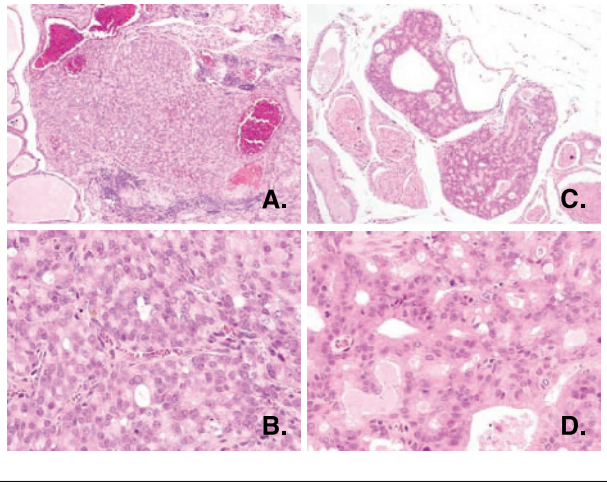

Figure 1. Distinct histological features of prostate cancers in ACI and F344 rats. Lesions of ACI rats (*A* and *B*) were characterized by high incidences of solid structures (*A*) and round, irregular nuclei (*B*). Lesions of F344 rats (*C* and *D*) were characterized by a papillary or cribriform pattern (*C*) and nuclei with angles and relatively regular sizes (*D*). Lesions in ACI rats were larger and invaded into flanking lobules showing their more malignant characteristics.

Table 2. Results of QTL analysis in F_2 intercross rats

Lesions used for linkage analysis	*Pcs1* (chromosome 19)		*Pcr1* (chromosome 2)	
	LOD scores	% Variance explained	LOD scores	% Variance explained
Any lesions in prostate				
Presence	2.9	10.6	0.4	1.7
Number	3.2	12.2	1.9	7.6
Area (mm^2)	0.4	1.5	3.7	16.2
Area ratio (%)	0.2	0.8	5.0	20.3
Lesions with a solid structure				
Presence	3.0	11.4	0.7	2.7
Number	2.8	10.7	1.1	4.2
Area (mm^2)	0.1	0.4	2.3	9.0
Area ratio (%)	0.2	0.7	2.9	11.4
Lesions with ACI-type nuclei				
Presence	5.0	17.6	0.5	2.0
Number	3.7	13.5	1.3	5.2
Area (mm^2)	0.4	1.7	2.7	11.4
Area ratio (%)	0.1	0.5	4.0	15.6

Linkage analysis was done using areas containing the three kinds of lesions, and a QTL was identified in the vicinity of *D2Rat161* (LOD scores of 3.6, 2.3, and 2.7, respectively; Table 2). The same QTL was mapped using the area ratio. Notably, this locus conferred ACI-dominant resistance to an increase in size, and the effect was clearer using areas with any lesion than with using areas with lesions characteristic to ACI rats. F_2 intercross rats with the ACI/ACI, ACI/F344, and F344/F344 genotypes at *D2Rat161* had area ratios of 0.8 ± 1.2% (mean ± SD), 0.9 ± 1.0%, and 2.5 ± 2.6%, respectively (Fig. 3). This locus, *prostate cancer resistance 1* (*Pcr1*), had a paradoxical effect on the size of prostate cancers (Fig. 2*B*). This region (*D2Rat26-D2Rat40*) corresponded to rat 2q23-q32.

Effect of bilateral testicular tumors on prostate cancers. The size of the ventral prostate was significantly smaller in F_2 intercross rats with bilateral macroscopic testicular tumors (0.24 ± 0.13 g, $n = 67$) than in those with single or no tumors (0.49 ± 0.42 g, $n = 51$, $P < 0.005$). However, the incidences and mean areas of tumorous lesions in the ventral prostate were not different (60 of 67 and 1.0 ± 1.4 mm^2 in the former rats, and 45 of 51 and 1.3 ± 1.6 mm^2 in the latter rats). The mean serum testosterone level at 30 months was 0.1 ± 0.2 ng/mL in rats with bilateral tumors and 1.3 ± 5.6 ng/mL (0.3 ± 0.4 ng/mL when two extremely high values were excluded) in rats without bilateral tumors. Although the presence of bilateral testicular tumors did not affect the incidence or area of tumorous/hyperplastic lesions in the prostate, two new QTLs were mapped when linkage analysis was done in rats without bilateral testicular tumors (Table 3). One QTL in the vicinity of *D20Rat3*, *Pcs2*, conferred an ACI-dominant increase in the incidence of lesions

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(LOD scores of 1.9-3.3; Fig. 2*C*). Another QTL in the vicinity of *D1Rat211*, *Pcr2*, conferred a paradoxical effect, ACI-dominant suppression of the area ratio (LOD scores of 3.9-4.3; Fig. 2*D*). *Pcs2* (*D20Rat41-D20Rat5*) and *Pcr2* (*D1Rat121-D15Rat45*) corresponded to rat 20p12-11 and 1p12-q11, respectively.

Oligonucleotide microarray analysis of genes within the loci. By oligonucleotide microarray analysis using the prostates of ACI and F344 rats at 8 weeks, 219 and 143 probes were found to be expressed higher and lower, respectively, at ≥1.8-fold in the ACI prostates. At 48 weeks, 371 and 154 probes were expressed higher and lower, respectively, in the ACI prostates. Forty-eight and 34 genes were expressed higher and lower both at 8 and 48 weeks (Supplementary Table S2). Among these genes, those located within the *Pcs1*, *Pcr1*, *Pcs2*, and *Pcr2* regions were RT1 class II (locus *DMa*; 2.1- to 3.0-fold), *membrane metalloendopeptidase* (*Mme*; 1.9- to 3.7- fold), and *Gstt2* (3.0- to 6.1-fold).

RT1 and *Gstt2* in *Pcs2* were found to have sequence polymorphisms in their oligonucleotide probes, which caused apparent expression difference in the microarray analysis (35). In contrast,

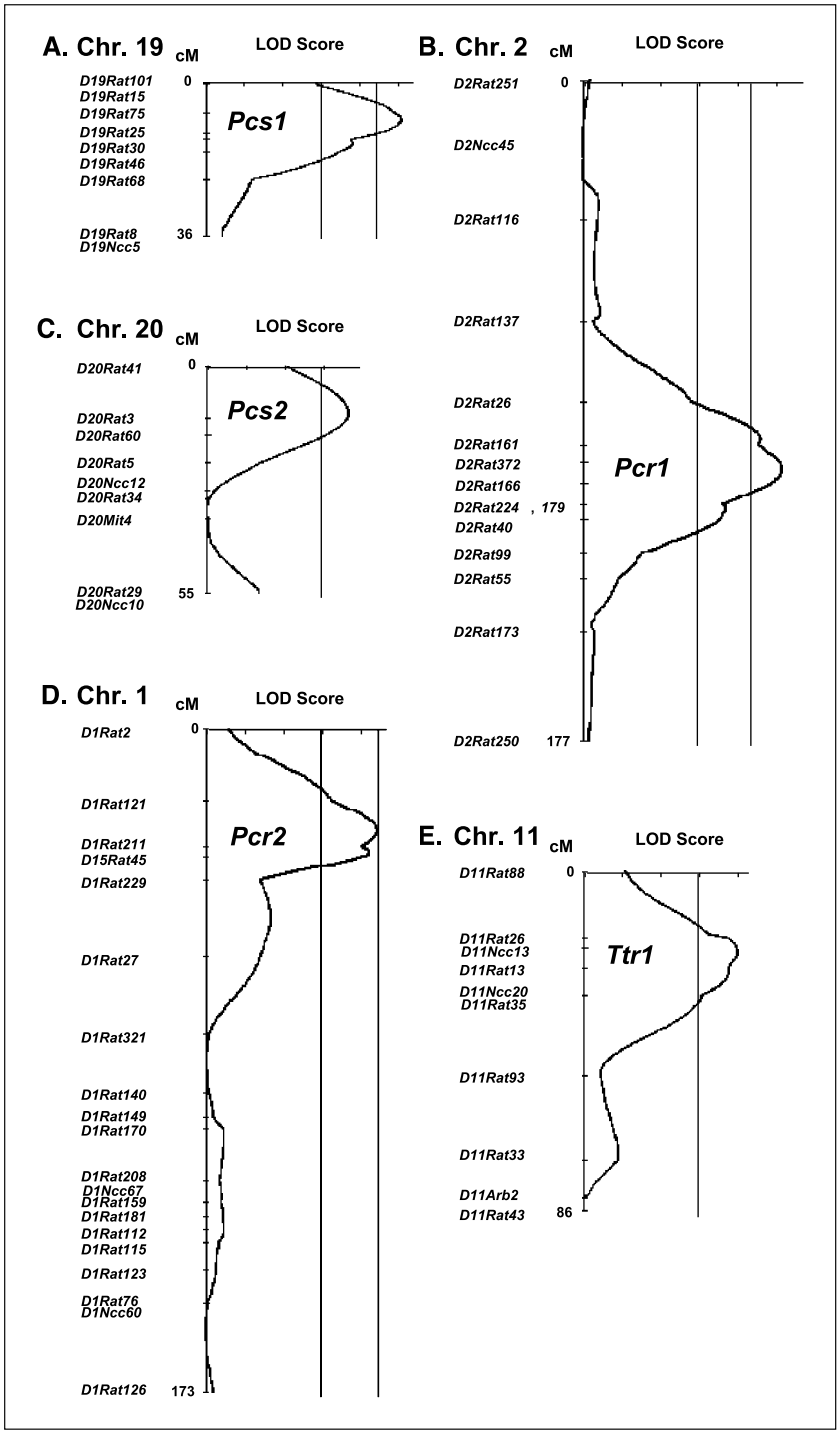

Figure 2. LOD score obtained for linkage with prostate lesions and testicular tumors. *A,* LOD score obtained by the presence of lesions with ACI-type nuclei in the 118 F_2 intercross rats. ACI allele in this locus dominantly promoted induction of the lesions. *B,* LOD score obtained by the area ratio of any lesions in the 118 F_2 intercross rats. ACI allele in this locus dominantly and paradoxically suppressed the growth of the lesions. *C,* LOD score obtained by the presence of lesions with ACI-type nuclei in the F_2 intercross rats with single or no testicular tumors. ACI allele in this locus dominantly promoted the growth of the lesions in this subgroup of rats. *D,* LOD score obtained by the area ratio of any lesions in the F_2 intercross rats with single or no testicular tumors. ACI allele in this locus dominantly and paradoxically suppressed the growth of the lesions in this subgroup. *E,* LOD score obtained by development of testicular tumors. The F344 allele in this locus promoted development of testicular tumors. *Dashed line,* Lander-Kruglark threshold for "significance" (LOD score of 4.3 in intercross), and "suggestive" (LOD score of 2.8 in intercross) linkage (32).

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Mme in *Pcr1* was confirmed to be expressed at higher levels in the ACI prostate (2.0- to 5.5-fold) by quantitative reverse transcription-PCR (RT-PCR; Table 4). The difference and its location in the *Pcr1* region suggested *Mme* as a candidate for *Pcr1*. In addition, we previously found that *Cdkn1a* (*p21*) on *Pcs2* was differentially expressed in the prostates of various rat strains,[4] and its high expression in F344 rats was confirmed by quantitative RT-PCR (1.5- to 4.5-fold, Table 4). Although the *Cdkn1a* difference could not be identified by the oligonucleotide microarray analysis, the difference and its location in the *Pcs2* region suggested *Cdkn1a* as a candidate for *Pcs2*.

Mapping for the other QTLs. Linkage analysis with the presence of testicular tumors was done in the 118 F_2 intercross rats, and one QTL was identified in the vicinity of *D11Ncc20*. An *F344* allele of this locus dominantly promoted the development of testicular tumors (LOD score of 3.9; Fig. 2*E*). The incidences were 71% (17 of 24) in rats with the ACI/ACI genotype and 94% (91 of 94) in rats with the ACI/F344 and F344/F344 genotypes. The QTL, *testicular tumor resistance 1* (*Ttr1*), explained 14% of the total variance.

One QTL, whose ACI allele semidominantly induced URA, was mapped in the vicinity of *D14Rat65* (LOD score of 2.9). URA was observed in 20% (10 of 49), 9% (7 of 77), 0% (0 of 56) of rats with ACI/ACI, ACI/F344 and F344/F344 genotypes, respectively. The QTL, *Ura1*, explained 13% of the total variance. No QTLs were mapped for the serum testosterone levels, the development of leukemia and that of s.c. tumors at 30 months.

Discussion

Prostate cancers in ACI rats occasionally progressed into invasive cancers, which were considered to model human clinically significant prostate cancers. In contrast, prostate cancers in F344 rats stayed as small lesions, if present, which was considered to model human indolent prostate cancers. By using these strains, two prostate cancer susceptibility and two resistance genes were mapped using F_2 intercross progeny of ACI and F344 rats. Dominant ACI alleles of *Pcs1* (chromosome 19) and *Pcs2* (chromosome 20) increased the incidence and the number of prostate lesions, and these genes were considered to be involved in the development of prostate cancers. In contrast, dominant ACI alleles of *Pcr1* (chromosome 2) and *Pcr2* (chromosome 1) paradoxically decreased the area and area ratio of lesions, and these genes were considered to suppress the growth of prostate cancers. A similar example that an allele of a susceptible strain confers a resistance has been reported (27). Even when these four genes were combined, these did not fully explain the genetic predisposition of ACI rats. It was therefore shown that susceptibility of ACI rats to prostate cancers is determined by the four genes mapped here and many other weak genes.

The effect of *Pcs2* and *Pcr2* was clearly observed in rats without bilateral macroscopic testicular tumors and masked in rats with such tumors. Testicular tumors of rats are known to be associated with a tendency towards feminization (37). Although serum testosterone levels are known to be highly variable (26), 30-month-old rats with bilateral macroscopic testicular tumors had lower testosterone levels in this study. Therefore, as a possible mechanism for the differential effects of *Pcs2* and *Pcr2*, involvement of different molecular pathways in the development of prostate cancers depending upon testosterone levels was considered. Because *Pcs2* promotes and *Pcr2* suppresses prostate cancers and many other unmapped genes are potentially involved, the incidence and area of prostate cancers were not different between rats with and without bilateral macroscopic testicular tumors. Temporal analysis on the development of prostate cancers, that of testicular tumors, and testosterone levels is necessary to clarify possible involvement.

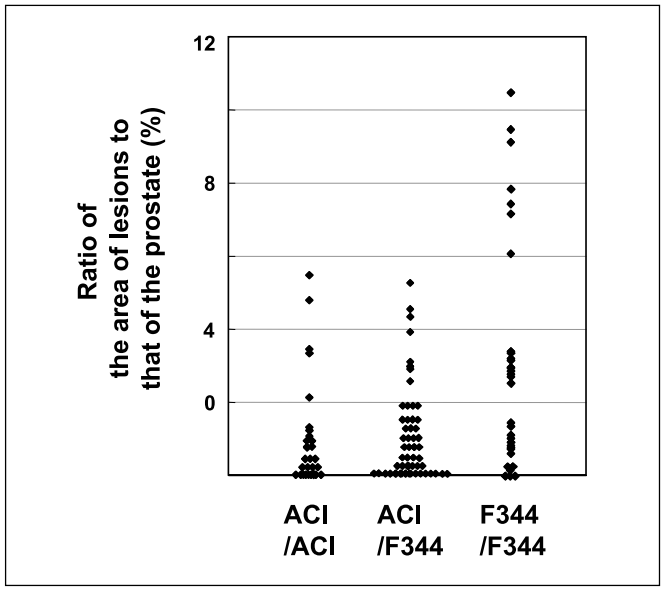

Figure 3. Effect of *Pcr1* locus on the area ratio of lesions. When F_2 intercross rats were classified by their genotype at *D2Rat161*, rats with the ACI allele(s) showed smaller area ratios.

By combining positional information obtained by linkage analysis and expression information obtained by oligonucleotide microarray analysis (38), two candidate genes were identified. GeneChip "Rat Genome U34A" covered 39 of 158 genes within the region of *Pcs1*, 77 of 308 in *Pcr1*, 86 of 191 in *Pcs2*, and 39 of 115 in *Pcr2*, accounting for 31% of the genes within the mapped loci. In addition, the responsible genes in the loci are not necessarily differentially expressed. Therefore, the combination strategy is effective only when candidate genes are obtained, and no good candidates were found for *Pcs1* and *Pcs2* in this study. Nevertheless, *Mme* (also called *Neutral endopeptidase*, *Cd10*), which showed higher expression in ACI rats, was considered as a good candidate for *Pcr1* that had a paradoxical effect. *Mme* expression was known to suppress growth of androgen-independent prostate cancer cells and was decreased in androgen-independent prostate cancers (39). Therefore, its higher expression in ACI was in accordance with its paradoxical effect. *Cdkn1a*, which showed lower expression in ACI rats, was a good candidate for *Pcs2*. *Cdkn1a* is a well-established negative regulator of the cell cycle, and a structural abnormality in its 5′ of promoter region has been identified.[4]

Chromosomal regions around *Pcs1*, *Pcr1*, *Pcs2*, and *Pcr2* corresponded to human 19p13, 4q28-31, and 16q21-22; 3q26-27, 13q12-14, 3q21-26, and 4q32; 6p21, 21q22, 22q11, and 10q21; and 6q23-25 and 5p15, respectively. As for *Pcs1*, linkage of Swedish familial prostate cancers to 19p13 (40) and frequent loss of heterozygosity in human prostate cancers (16q22; refs. 41, 42) are

[4] Yamashita et al., submitted for publication.

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Table 3. QTLs for prostate lesions in subgroups classified by testicular tumors and testosterone levels

Population	Variable for linkage analysis	*Pcs1* (chromosome 19)		*Pcr1* (chromosome 2)		*Pcs2* (chromosome 20)		*Pcr2* (chromosome 1)	
		LOD scores	% Variance explained	LOD scores	% Variance explained	LOD scores	% Variance explained	LOD scores	% Variance explained
All F2 intercross rats (n = 118)	Presence of any lesions	2.9	10.6	0.4	1.7	0.4	1.9	0.9	3.6
	Presence of lesions with ACI-type nucleus	5.0	17.6	0.5	2.0	1.9	8.6	0.9	3.4
	Area ratio of any lesions	0.2	0.8	5.0	20.3	0.5	2.0	0.6	2.3
	Area ratio of lesions with ACI-type nucleus	0.1	0.5	4.0	15.6	1.5	6.7	0.8	3.1
Rats with no or unilateral testicular tumors (n = 51)	Presence of any lesions	0.8	7.1	0.7	6.3	1.9	17.2	1.6	17.5
	Presence of lesions with ACI-type nucleus	1.6	13.7	0.6	5.7	3.4	32.4	1.2	12.0
	Area ratio of any lesions	1.3	11.1	2.0	22.9	1.1	10.0	3.9	33.0
	Area ratio of lesions with ACI-type nucleus	0.8	6.8	1.3	11.1	1.2	10.6	4.3	35.4
Rats with bilateral testicular tumors (n = 67)	Presence of any lesions	2.7	17.2	0.6	6.2	1.6	14.0	0.5	3.2
	Presence of lesions with ACI-type nucleus	3.9	24.5	1.3	11.0	1.2	10.5	1.0	6.5
	Area ratio of any lesions	0.6	3.9	3.4	22.9	1.4	9.1	0.7	6.0
	Area ratio of lesions with ACI-type nucleus	0.5	3.1	2.8	18.3	1.2	8.0	0.5	3.7

reported. Introduction of human chromosome 19p13 into both rat and human prostate cancer cells suppressed tumorigenicity in nude mice (43). As for *Pcr1*, its candidate, *Mme*, is located on human 3q25. Frequent deletion of human 13q in prostate cancer is reported (44). As for *Pcs2*, in addition to *Cdkn1a* on human 6p21, polymorphisms of *Gstt1* on human 22q11 were reported to give differential risks to prostate cancers (3). As for *Pcr2*, *Srd5a1*, whose polymorphism is associated with the metastatic potential of prostate cancers (36), was located on *Pcr2*.

The genes involved in the development of testicular tumors (*Ttr1*) and URA (*Ura1*) were successfully mapped on chromosomes 11 and 14, respectively. These regions correspond to human chromosome 21q21-22 and human chromosome 4q11-12, respectively. Linkage analysis is almost impossible in humans for diseases with low penetrance, such as URA, and positional information obtained here would be useful if candidate genes are obtained from their functions.

In summary, we were able to map four susceptibility and resistance genes involved in the prostate cancer susceptibility of ACI rats. A complex interaction of multiple susceptibility genes and effects of testicular tumors in prostate cancer susceptibility was shown. Combining the data with those obtained by oligonucleotide microarray, *Mme* and *Cdkn1a* were identified as candidates.

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Table 4. Quantitative RT-PCR analyses for genes within the QTLs

Gene	Location	Gene expression levels copy number/β-*actin* ($\times 10^4$, mean, n = 3)				
		8 wks			48 wks	
		ACI	F344	(F344 × ACI) F_1	ACI	F344
Mme	*Pcr1* chromosome 2q31	1,413	255	976	2,608	1,328
Cdkn1a	*Pcr2* chromosome 20p12	9.9	15.1	16.6	8.3	37.7

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