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Chromosomal Mapping of Genes Controlling Development, Histological Grade, Depth of Invasion, and Size of Rat Stomach Carcinomas[1]

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ABSTRACT

Rat stomach cancers induced by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) are widely used as a model of differentiated-type human stomach cancers. ACI/N (ACI) rats are susceptible and BUF/Nac (BUF) rats are resistant to MNNG-induced stomach carcinogenesis, and the presence of an autosomal gene with a dominant BUF allele has been suggested. In this study, we performed a carcinogenicity test by giving MNNG in drinking water to 117 male ACI × (ACI×BUF)F_1 backcross rats. Each of 100 effective rats was diagnosed for its "carcinoma development" and when it was bearing stomach carcinoma(s), for histological grade, depth of invasion, and size and number of tumors. Carcinoma development was diagnosed based both on the age of the rat and on the presence of stomach carcinoma(s). Linkage analysis was performed with the genotypes of 161 loci, covering 1637 cM of the rat genome. Contrary to our original expectations, the most influential gene was the one on chromosome (chr.) 15, *Gastric cancer susceptibility gene 1* (*Gcs1*), which confers susceptibility to stomach carcinogenesis (LOD, 3.8) with a dominant BUF allele by promoting conversion from adenomas to carcinomas. Two resistance genes on chr. 4 and chr. 3, *Gastric cancer resistance gene 1* (*Gcr1*) and *Gcr2*, were shown to confer dominant resistance (LOD, 2.7 and 2.6, respectively). *Gcs1*, *Gcr1*, and *Gcr2* exerted additive effects on the development of stomach carcinomas. A gene on chr. 16, *Gcr3*, was indicated to reduce the depth of invasion (LOD, 2.2) and sizes of tumors (LOD, 1.9). No linkage was obtained using the number of tumors. These findings show that the coordinate effect of a susceptibility gene, *Gcs1*, and two resistance genes, *Gcr1* and *Gcr2*, is responsible for the development of MNNG-induced stomach carcinomas and that *Gcr3* is responsible for the growth of a stomach carcinoma, reflected in the depth of invasion and in the tumor size.

INTRODUCTION

Investigations into the genes responsible for cancer predisposition are important. Identification of high-risk populations for a type of cancer can lead to its prevention and its diagnosis and treatment in early stages. For example, the discovery of *BRCA1* and *BRCA2* made it possible to identify high-risk family members for breast cancers, and genetic tests have been implemented (1). Clarification of the involvement of mismatch repair genes, such as *hMSH2* and *hMLH1*, in hereditary non-polyposis colorectal cancer syndromes has also changed clinical approaches to high-risk patients (2). In the case of stomach cancer, which is the major cause of cancer death in Asian countries, genetic factors have also been implicated (3). Recently, germ-line mutations of the *E-cadherin* gene were found in families from New Zealand, in which early-onset and highly malignant diffuse-type stomach cancers are clustered (4). However, the gene(s) responsible for predisposition to differentiated-type stomach cancers still remain unknown. Moreover, linkage analysis using human families is facing critical limitations—decreasing numbers of family members and the heterogeneity of responsible genes among families.

The rat stomach carcinoma induced by administration of MNNG[3] in drinking water provides a good model for human stomach cancer of a differentiated type (5). The histology of stomach carcinomas induced in the model closely resembles that of human differentiated-type stomach carcinomas, and their carcinogenic processes are modulated by various promoting and preventive agents in a way similar to that observed in human populations (6–8). Differences in the susceptibility to MNNG-induced stomach carcinomas have been known among rat strains (9–12). Stomach carcinomas are induced in the ACI/N (ACI) strain at a rate of ~80%, and in the BUF/Nac (BUF) strain at ~20% (11). The incidences of stomach carcinomas in F_1 and F_2 rats of ACI and BUF rats indicated involvement of an autosomal gene whose BUF allele is dominant over the ACI allele (11).

As the mechanism of the different susceptibilities, different levels of cell proliferation in response to chronic mucosal damage by MNNG, superimposed with a strong inflammatory reaction, have been implicated (13). Whereas ACI rats show a large increase in the cell proliferation rate after MNNG treatment, BUF rats show only a small increase. It is likely that a high rate of cell proliferation leads to a high rate of mutations, and finally to a high incidence of stomach carcinomas. MNNG is a direct-acting carcinogen, excluding differences in the capacity of metabolic activation as a mechanism for the different cancer susceptibilities between ACI and BUF rats. Moreover, DNA adduct levels in the pylorus of the two strains are the same (5), excluding differences in the local distribution of MNNG as a mechanism. These previous findings suggest that the mechanisms involved in the different susceptibilities between the two strains are not restricted to MNNG-induced stomach carcinogenesis.

To identify the gene(s) involved in the different susceptibility between ACI and BUF rats, where involvement of an autosomal dominant gene of the BUF type has been suggested, we produced 117 male ACI × (ACI×BUF)F_1 backcross rats in this study, and performed a carcinogenicity test for 80 weeks. "Carcinoma development" in individual rats and, when a rat was bearing one or more tumors, the histological grade of malignancy, depth of tumor invasion, tumor size, and number of tumors per rat were determined. The results of linkage analysis are reported.

MATERIALS AND METHODS

Carcinogenicity Test and Histological Examination. ACI and BUF rats were purchased from Japan Clea (Tokyo, Japan). F_1 rats were produced by mating female ACI rats with male BUF rats, and backcross rats were produced by mating female ACI rats with male F_1 rats.

ACI, BUF, and backcross rats were caged in groups of three to four rats and fed Oriental MF diet (Oriental Yeast, Tokyo, Japan). The cages were kept in an air-conditioned room at 25°C and 55% humidity with 12-h light and dark cycles. The rats were administered 83 mg/l MNNG in drinking water *ad libitum* from the age of 8 weeks through the age of 40 weeks. MNNG was

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[3] The abbreviations used are: MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; ACI, ACI/N strain; BUF, BUF/Nac strain; QTL, quantitative trait locus; *Gcs*, gastric cancer susceptibility gene; chr., chromosome; *Gcr*, gastric cancer resistance gene.

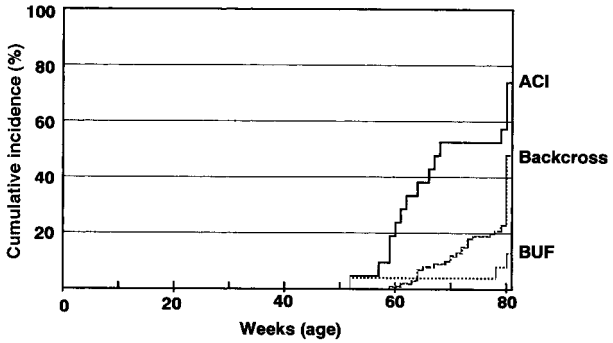

Fig. 1. Cumulative incidence of stomach cancers in ACI, BUF, and backcross rats. Rats that survived 52 weeks or more were counted as effective rats and are shown.

purchased from Sigma-Aldrich (St. Louis, MO), and a fresh 830 mg/l (deionized water) stock solution was prepared once a week. The stock solution was diluted 10-fold with deionized water twice a week. Rats that became moribund or reached 80 weeks of age were sacrificed.

The stomach was opened along the greater curvature, and sections of the glandular stomach were prepared by cutting along the sagittal axis. When a macroscopic tumor was observed, the tumor was cut into two pieces, one of which underwent histological examination and the other was kept frozen. When a macroscopic tumor was absent, slices were made at 2-mm intervals underwent histological examination. Histological examination was performed by an experienced pathologist (M. T.), and when stomach carcinoma was present, its histological grade of malignancy, depth of tumor invasion, maximal diameter, and number of tumors per rat were determined (14). When two or more carcinomas were present in a rat, the most malignant phenotype from one of the carcinomas was adopted as the phenotype of the rat.

The liver and/or tail of each rat was kept frozen, and DNA for genotyping was extracted by serial extraction with phenol and chloroform (15).

Genotyping. Genotypes of 161 loci, consisting of 146 microsatellite markers, 5 B1-RDA markers (16), and 10 AP-RDA markers (17), were determined for all of the 100 effective rats. Distances between markers summed up to 1637 cM, covering almost the entire genome of the rat. The average of the distances between two markers was 11.6 cM.

Microsatellite primers were synthesized by Sawady Technology (Tokyo, Japan) based on previous reports (18), or purchased from Research Genetics (Huntsville, AZ; Ref. 19). Annealing temperatures of 60°C and 55°C and Mg^{2+} concentrations of 1.5 and 2.5 mM were first tested for each pair of primers, and PCR for genotyping was performed in the optimized condition using 25 ng of template DNA. PCR products were run in 3% NuSieve gels or in 6% polyacrylamide gels.

Genotyping with B1-RDA and AP-RDA markers was performed as reported previously (16, 17). "B1-amplicons" and "AP-amplicons" were prepared by amplifying 100 ng of genomic DNA with an appropriate B1- or AP-primer and then dot-blotted onto a nylon membrane after denaturation. The filters were hybridized with B1-RDA or AP-RDA markers, which had been labeled with random hexamers and the Klenow fragment (Multiprime; Amersham-Pharmacia), and purified by gel filtration chromatography (Microspin; Amersham-Pharmacia). Prehybridization, hybridization, wash, and signal detection were performed as reported (16, 17). All of the markers used were selected on the basis that they give signals with BUF but not with ACI.

Linkage Analysis. A genome map for the backcross rats was calculated with Mapmaker/EXP software (20). A genome-wide survey for QTLs was performed with the Mapmaker/QTL software (20). For the carcinoma development phenotype, a rat was given a digit of 0 or 2, depending on the diagnosis. For the "histological grade" and "depth of tumor invasion" phenotypes, a rat was given scores ranging from 1 to 5. For the "tumor size" phenotype, the maximal diameter of the carcinoma (in mm) in histological sections was used. To assess the effects of the genotypes, χ^2 tests and *t* tests for unequal distribution were performed.

RESULTS

Carcinogenicity in Inbred and Backcross Rats. The cumulative incidences of the stomach carcinomas in ACI, BUF, and backcross rats are shown in Fig. 1. The first stomach carcinoma in ACI rats was observed at the age of 52 weeks. Therefore, backcross rats that survived 52 weeks or more and whose stomach histology was available were counted as effective animals. Deaths of the ACI and backcross rats before 52 weeks were caused mainly by hydronephrosis and resultant renal failure, which is known to take place in ACI rats at an incidence of 5–20% (21, 22). Deaths of the ACI, BUF, and backcross rats after 52 weeks were due to stomach carcinomas, duodenal sarcomas, pneumonia, and other causes. Incidences of duodenal sarcomas are known to be in the same range in ACI and BUF rats (11). Similar to previous studies (10, 11), stomach carcinomas were observed in 16 of 21 effective ACI rats (76%) and 3 of 22 effective BUF rats (14%). In the backcross rats, 48 of 100 effective rats (48%) developed stomach carcinomas (Table 1).

Linkage with Carcinoma Development. To diagnose the carcinoma development phenotype of the backcross rats, the rats were classified by the size of stomach carcinomas that they bore and by their age when they were sacrificed (Table 1). The diagnosis on their susceptibility was made by mimicking the sizes and ages observed in inbred strains. Because it was impossible to make a definitive classification before performing linkage analyses, we tested 12 combinations of three criteria for the susceptible rats (S1, S2, and S3) and four criteria for the resistant rats (R1, R2, R3, and R4), as shown in Table 1.

For the rats that survived the full-term of the carcinogenicity test (79–80 weeks of age), rats with stomach carcinomas of a large size could be diagnosed as "susceptible" and those without a carcinoma could be diagnosed as "resistant." The diameter of the smallest carcinoma in the ACI rats at this period was 5 mm in this study. Therefore, a backcross rat with a carcinoma 3 mm in diameter was not classified as susceptible, and those with carcinomas 4 mm in diameter were classified as susceptible in one criterion (S3) and not in the others (S1, S2). For the rats that were sacrificed at early periods of the carcinogenicity test, only those with a stomach carcinoma could be diagnosed as susceptible, but those without a carcinoma could not be diagnosed as susceptible or resistant because they might have developed a stomach carcinoma if they had survived the full-term. To explore what age was appropriate to mark "early periods," we tested different criteria for resistant rats (R1–R4).

Linkage analysis was performed using these 12 combinations of the carcinoma development phenotype, and three loci were found to give LOD scores >2.0 with at least one of the 12 combinations (Table 2 and Fig. 2). Because criteria S1, S2, and S3 gave similar LOD score curves, results with S3 and R1-R4 are shown in Fig. 2. The most influential gene, *Gcs1*, was mapped on chr. 15 with the highest LOD score of 3.8 (criterion R1S3; $n = 69$), and was found to have a paradoxical effect on the

Table 1 *Distribution of the ages of backcross rats and the sizes of stomach cancers*

Rats that survived 52 weeks or more were counted as effective rats ($n = 100$). In addition to histological diagnosis, rats with carcinomas were classified by the maximal diameters of their carcinomas. Sizes of adenomas ranged from 0.5 to 3 mm. Tumors that could not be diagnosed as malignant or benign were classified as "borderline" tumors. Three kinds of criteria for susceptible rats (S1, S2, and S3) and four kinds of criteria for resistant rats (R1, R2, R3, and R4) were set, and 12 kinds of carcinoma development phenotypes were produced by combining S1–S3 and R1–R4. S1, s1 ($n = 44$); S2, s1–s2 ($n = 45$); S3, s1–s3 ($n = 47$); R1, r1 ($n = 22$); R2, r1–r2 ($n = 25$); R3, r1–r3 ($n = 26$); and R4, r1–r4 ($n = 31$).

	No. of rats in each group						
Histology	Age (weeks) 52–58	59–63	64–68	69–73	74–78	79–80	Total
Adenocarcinoma							
>5 mm		1^{s1}	5^{s1}	8^{s1}	1^{s1}	23^{s1}	38
≤5 mm					2^{s1}	1^{s2}	3
≤4 mm		1^{s1}	1^{s1}	1^{s1}		2^{s3}	5
≤3 mm		1^{s1}				1	2
Borderline			1	4	1^{r3}	3^{r2}	9
Adenoma	5		4	2	1^{r1}	11^{r1}	23
No tumor	3	2	4^{r4}	1^{r4}	2^{r1}	8^{r1}	20
Total	8	5	15	16	7	49	100

Table 2 *Summary of the results of QTL mapping*

Susceptibility was scored as 2 (susceptible) or 0 (resistant). Histological grade and depth of tumor invasion were classified arbitrarily. Tumor size was measured as tumor diameter in mm. LOD scores greater than 2.0 are underlined.

		Chr. 3 *D3Rat55*		Chr. 4 *Ampp*		Chr. 15 *D15Rat102*		Chr. 16 *D16Rat17*	
Trait	Population used	LOD score	Effect	LOD score	Effect	LOD score	Effect	LOD score	Effect
Carcinoma development	R1S3 (n = 69)	1.88	−0.64	2.38	−0.74	3.84	0.95	0.10	−0.16
	R2S3 (n = 72)	2.41	−0.72	2.48	−0.74	3.84	0.95	0.11	−0.16
	R3S3 (n = 73)	2.59	−0.75	2.70	−0.76	3.41	0.87	0.05	−0.11
	R4S3 (n = 78)	1.83	−0.63	1.67	−0.61	2.67	0.77	0.00	0.00
Histological grade	EA[a] (n = 100)	0.90	−0.60	0.89	−0.58	2.22	0.96	0.02	−0.09
	ET (n = 80)	0.48	−0.39	0.26	−0.29	3.55	1.13	0.01	−0.05
	EC (n = 48)	0.10	0.11	0.64	0.30	0.01	0.04	1.11	−0.37
	FA (n = 49)	0.76	−0.80	0.87	−0.85	2.11	1.42	1.02	−0.95
	FT (n = 41)	0.44	−0.55	0.48	−0.57	2.98	1.44	1.08	−0.87
	FC (n = 27)	0.07	0.14	0.54	0.39	0.34	−0.36	1.80	−0.65
Depth of tumor invasion	EA (n = 100)	0.55	−0.21	0.74	−0.31	0.36	0.37	0.22	−0.29
	ET (n = 80)	0.20	−0.23	0.08	−0.14	1.20	0.56	0.33	−0.30
	EC (n = 48)	0.16	0.25	0.13	0.24	0.00	0.03	1.27	−0.68
	FA (n = 49)	0.33	−0.50	0.21	−0.40	1.06	0.88	1.69	−1.13
	FT (n = 41)	0.07	−0.20	0.00	−0.05	1.13	0.79	2.17	−1.13
	FC (n = 27)	0.27	0.46	0.56	0.66	0.03	−0.19	2.33	−1.31
Tumor size	EA (n = 100)	0.18	−1.65	0.05	−0.86	1.42	4.55	0.60	−3.04
	ET (n = 80)	0.05	−1.02	0.01	0.34	1.30	5.06	0.61	−3.55
	EC (n = 48)	0.17	2.66	0.38	4.13	0.10	2.13	1.50	−7.62
	FA (n = 49)	0.11	−2.30	0.07	−1.78	1.32	7.59	2.07	−9.63
	FT (n = 41)	0.03	−1.34	0.00	−0.45	1.09	7.87	1.92	−10.30
	FC (n = 27)	0.19	4.36	0.26	5.30	0.07	3.17	1.77	−12.80

[a] EA, all effective rats; ET, rats with tumors; EC, rats with carcinomas; FA, full-term rats; FT, full-term rats with tumors; FC, full-term rats with carcinomas.

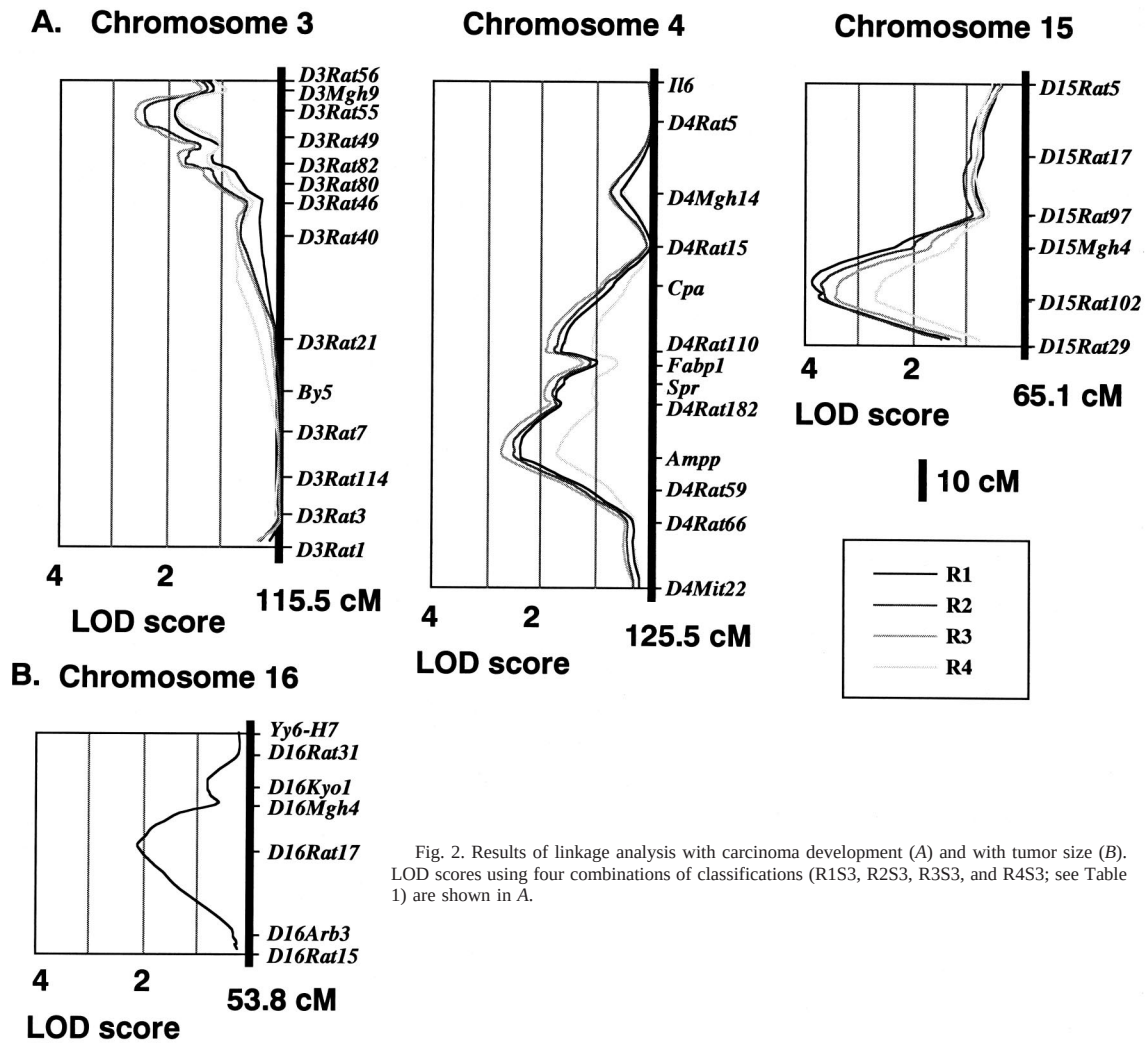

Fig. 2. Results of linkage analysis with carcinoma development (*A*) and with tumor size (*B*). LOD scores using four combinations of classifications (R1S3, R2S3, R3S3, and R4S3; see Table 1) are shown in *A*.

Table 3 *Coordinate effect of Gcs1, Gcr1, and Gcr2 on carcinoma development*

Incidences of stomach cancers are shown in each group of rats classified by the genotypes of *Gcs1* (*D15Rat102*), *Gcr1* (*Ampp*), and *Gcr2* (*D3Rat55*). Criterion R3S3 was used to diagnose susceptible and resistant rats in this Table.

Gcs1 (*D15Rat102*)	*AA*				*AB*			
Gcr1 (*Ampp*)	*AA*		*AB*		*AA*		AB	
Gcr2 (*D3Rat55*)	*AA*	*AB*	*AA*	*AB*	*AA*	*AB*	*AA*	*AB*
Effective rats (*n*)	12	3	4	13	13	12	5	11
Rats with stomach cancer (*n*)	8	0	2	3	13	12	5	4
Incidence (%)	67	0	50	23	100	100	100	36

Table 4 *Classification of the backcross rats by the histological grade and effect of Gcs1*

BUF allele in *Gcs1* (*D15Rat102*) increased the incidence of stomach cancer, mainly by promoting conversion from adenomas to carcinomas.

		All effective rats			Rats surviving full term		
			D15Rat102			*D15Rat102*	
Histology	Score	Total	*AA*	*AB*	Total	*AA*	*AB*
No tumor		20	11	9	8	5	3
Adenoma	1	23	17	6	11	9	2
Borderline	2	9	5	4	3	2	1
Adenocarcinoma		48	14	34	27	6	21
Well-differentiated	3	28	9	19	14	2	12
Moderately differentiated	4	18	4	14	11	3	8
Poorly differentiated or signet-ring cell	5	2	1	1	2	1	1
Total		100	47	53	49	22	27

development of stomach carcinomas. Despite the fact that the BUF rat is resistant as an inbred strain, the BUF allele at *Gcs1* conferred dominant susceptibility to MNNG-induced stomach carcinogenesis. Whereas 13 of 30 rats with the *AA* genotype (43%) at *Gcs1* (represented by *D15Rat102*) developed stomach carcinomas, 34 of 39 rats with the *AB* genotype (87%) developed carcinomas ($P = 0.0001$; Table 3).

Two genes, *Gcr1* and *Gcr2*, were mapped on chr. 4 and chr. 3 with peak LOD scores of 2.7 and 2.6, respectively, using criterion R3S3. The BUF alleles at these two loci were shown to confer dominant resistance. For *Gcr1* (represented by the marker, *Ampp*; Table 3), 33 of 40 rats with the *AA* genotype (83%) developed stomach carcinomas, whereas 14 of 33 rats with the *AB* genotype (42%) developed carcinomas ($P = 0.0004$). For *Gcr2* (represented by *D3Rat55*), 28 of 34 rats with the *AA* genotype (82%) developed stomach carcinomas, whereas 19 of 39 rats with the *AB* genotype (49%) developed carcinomas ($P = 0.003$). When *Gcr1* and *Gcr2* were combined, 21 of 25 rats (84%) with the *AA* genotypes at both loci developed stomach carcinomas, whereas 7 of 24 rats (29%) with the *AB* genotypes at both loci developed carcinomas ($P = 0.0002$). The effects of the two genes on the development of stomach carcinomas were considered as additive. *Gcs1* also showed an additive effect with *Gcr1* and *Gcr2*, but in an opposite direction. All of the 30 rats (100%) with the susceptible genotype at *Gcs1* (*AB*) and at either *Gcr1* or at *Gcr2* (*AA*) developed stomach carcinomas.

Linkage with the Histological Grade. All of the 80 rats with stomach tumors were given a score based on the histology of the tumors they bore (Table 4). Linkage analysis with the score showed a strong linkage with chr. 15, which was considered to be the same with *Gcs1* for the rats with tumors (LOD, 3.6; $n = 80$) but not for those with carcinomas ($n = 48$; Table 2). This indicated that *Gcs1* exerts its effect mainly at the stage of conversion from an adenoma to a carcinoma but that it does not have an effect on the progression of the histological grade of a carcinoma (see "Discussion"). When the backcross rats were classified by the genotypes of *Gcs1* (represented by *D15Rat102*), a major difference was found in the number of rats with an adenoma (Table 4). The LOD score obtained for the rats with tumors (ET group) was 3.6, whereas that obtained for the full-term rats with tumors (FT group) was 3.0.

Linkage with the Depth of Tumor Invasion and Size of Tumor. Linkage analysis was also performed with the depth of tumor invasion and with the size of tumor (Fig. 3*A*). Using the full-term rats, we found that the two phenotypes were linked to the same locus on chr. 16, designated as *Gcr3*, although the LOD scores were not definitive (Table 2).

The depth of tumor invasion was linked to *Gcr3* with a LOD score of 2.2 for full-term rats with a tumor (FT group) and 2.3 for full-term rats with a carcinoma (FC group; Table 2). For the FT group, the average score of depth of tumor was 3.3 in rats with the *AA* genotype in *Gcr3* (represented by *D16Rat17*), and 2.2 in those with the *AB* genotype (Table 5). The BUF allele at *Gcr3* was considered to reduce the depth of tumor invasion by a score of 1.1 in Table 5.

The average diameter of tumors was also linked to *Gcr3*, with LOD scores of 2.1 (all full-term rats; FA group), 1.9 (FT group), and 1.8 (FC group; Table 2). For the FC group, the average diameter was 21 mm in rats with the *AA* genotype in *Gcr3*, and 8 mm in those with the *AB* genotype (Fig. 3*B*). The BUF allele at this locus was considered to reduce the diameter of carcinomas by an average of 13 mm. The average tumor diameters were significantly different between the rats with the *AA* genotype and those with the *AB* genotype, for rats in the FC group ($P = 0.009$) or those in the FT group ($P = 0.007$).

Linkage with the Number of Tumors. Among the 100 effective rats, 72 rats developed one tumor, and 8 rats developed two tumors.

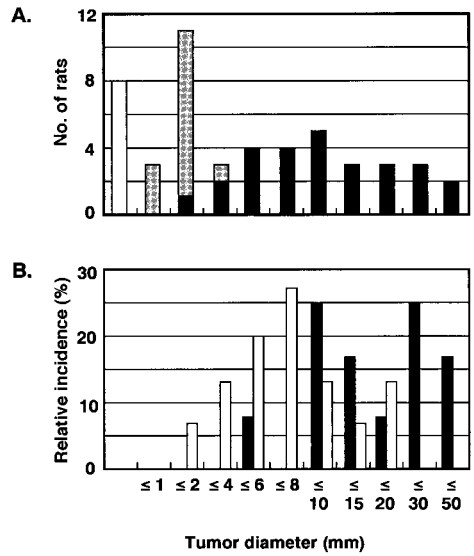

Fig. 3. Distribution of tumor sizes in full-term rats and the effect of *D16Rat17*. *A*, numbers of rats *versus* the size of tumors. Histological diagnosis of the tumors are shown: □, no tumors; ▒, adenomas; ■, carcinomas; *B*, effect of *Gcr3*, represented by *D16rat17*, on the size of carcinomas (n = 27). □, *AB*; ■, *AA*.

Table 5 *Classification of the backcross rats by the depth of tumor invasion and effect of Gcr3*

BUF allele in *Gcr3* (*D16Rat17*) was shown to reduce the depth of tumor invasion in the full-term rats.

		All effective rats			Rats surviving full term		
			D16Rat17			*D16Rat17*	
Depth	Score	Total	*AA*	*AB*	Total	*AA*	*AB*
No tumor		20	9	11	8	2	6
Mucosa	1	11	4	7	5	0	5
Submucosa	2	35	14	21	18	4	14
Muscularis propria	3	18	7	11	8	4	4
Subserosa	4	10	4	6	6	3	3
Serosa exposed	5	6	5	1	4	4	0
Total		100	43	57	49	17	32

Although linkage analysis was performed using the number of stomach carcinomas per rat, no linkage was observed.

DISCUSSION

We mapped one dominant susceptibility gene, *Gcs1*, and three dominant resistance genes, *Gcr1*, *Gcr2*, and *Gcr3*, involved in MNNG-induced stomach carcinogenesis. The involvement of multiple genes was contrary to our initial expectations of "a single dominant resistance gene" model, which had been proposed simply based on the carcinoma incidences in F_1 and F_2 populations. Other, more complicated models had not been excluded. Actually, the interaction of the four genes identified in this study explains the carcinoma incidences in the backcross rats in this study. Complex interaction of multiple genes has been reported in the mouse liver cancer model after treatment with urethane (23). Although it is expected that multiple genes are also involved in human cancer susceptibility, each gene with a relatively low penetrance, it is difficult to identify those genes in human families. An efficient approach would be to identify those genes in animal models and then to test the involvement of the genes in a human population.

Rat chr. 15 around *Gcs1* is known to have synteny with human chr. 13q (24, 25), where the endothelin receptor type B (*Ednrb*) is located. Rat chr. 4 around *Gcr1* has synteny with human 2p13, and it is speculated that the *Msh6* gene, encoding the G/T mismatch-binding protein, is located in the region. Although MNNG-induced stomach cancers do not display microsatellite instability (26), the *Msh6* gene is one of the candidate genes for *Gcr1*. The *MSH2* gene is also located on 2p in humans, but rat *Msh2* was mapped to chr. 6 (27), ruling out *Msh2* as a candidate for *Gcr1*. Rat chr. 3 around *Gcr2* has synteny with human 9q34 and harbors the prostaglandin G/H synthase gene (*Cox1*) and prostaglandin D2 synthase gene. *Cox1* is known to be involved in the cytoprotection of gastric mucosa and to be expressed in the stem cells of the pylorus (28). Considering that the suggested mechanism for the different susceptibilities between the two rat strains is the difference in the rates of cell proliferation in response to mucosal damage, *Cox1* is a candidate for *Gcr2*. Rat chr. 16 around *Gcr3* has synteny with human chr. 4q or 8p, where loss of heterozygosity is reported in human stomach cancers (29).

Gcs1 is the major gene that controls the development of carcinomas. The histological grade of tumors was also linked to *Gcs1* in rats with tumors, but the linkage was much weaker in rats with only carcinomas. This suggested that *Gcs1* is mainly involved in the conversion from adenomas to carcinomas. The distribution of the histological grades of the tumors in the rats classified by the genotype of *Gcs1* also supports this idea. Linkage of the histological grade with *Gcs1* was observed both in the rats of all experimental periods and in the full-term rats. This indicated that the effect of *Gcs1* is expressed from early periods to the end of the carcinogenicity test. On the other hand, linkage of the depth of tumor invasion and tumor size with *Gcr3* was observed only in the full-term rats, not in the rats of all experimental periods. This was considered because these two parameters change as a tumor grows.

Although LOD scores obtained for *Gcr1*, *Gcr2*, and *Gcr3* exceeded 1.9, the criterion for "suggested linkage" in backcross rats, they did not exceed 3.3, the criterion for "definitive linkage" (30). After we found that as many as four genes were involved, we considered the number of effective rats, 100, relatively small. However, classification of the backcross rats by the genotypes of one of the four genes clearly showed significant differences in phenotypes. The facts that both the development of carcinoma and histological grade were linked to *Gcs1* and that both depth of tumor invasion and sizes of tumors were linked to *Gcr3* further indicate that the possibility of false positives is very low.

In summary, we mapped three genes involved in the completion of malignant transformation of a stomach epithelium cell and one gene involved in the growth of the completed cancer cell. The suggested mechanism for the different susceptibilities between ACI and BUF leads us to expect that some of the four genes are also involved in human cancer susceptibility.

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