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New outbred colony derived from *Mus musculus castaneus* to identify skin tumor susceptibility loci

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Abstract

Susceptibility to tumor development varies among mice strains. Using inbred NIH and wild-derived outbred *Mus spretus* backcrosses, skin cancer-susceptibility loci were mapped [1][2], and *Skts13* was identified as the *Aurka* gene using a conventional linkage in conjunction with haplotype analysis [3].

In the present study, we examined another wild-derived outbred *Mus musculus castaneus* (*M.m.castaneus*) in which 10.3% of the analyzed SNPs showed heterogeneity among the colony. All mice examined were completely resistant to the two-stage skin carcinogenesis protocol using 7.12-dimethylbenz(a)anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA), and this resistant phenotype was dominant when we crossed them with the highly susceptible strain FVB. By scanning F1 backcross progeny between *M.m.castaneus* and FVB, we found a highly significant linkage for tumor multiplicity on Chromosome 4, which was overlapped with the *Skts-fp1* locus, found in the previous study using FVB and PWK cross [4]. The linkage was observed in all pedigrees from the five F1 founders, while the linkage for papilloma size on Chromosome 4 was mapped only in pedigrees from founders 1 and 2. By scanning the whole Chromosome 4 of the five F1 founders, founder1, 2-specific haplotype block was found between D4Mit293 (20.6 Mb) and D4Mit171 (22.4 Mb).

In this study we exploited the outbred nature of *M.m.castaneus* stock to identify a haplotype contributing to papilloma size on mouse chromosome 4. These data illustrate the strength of using outbred mice in identification of the genetic component of a mouse complex trait such as the skin cancer-susceptibility phenotype.

Keywords

Two stage skin carcinogenesis; Cancer susceptibility; *Mus musculus castaneus*; Outbred colony; Linkage disequilibrium mapping

Introduction

The effects of cancer-predisposing genes were discerned through studies of cancer occurrence in human families. In rare cases, the germ-line transmission of a defective allele at the tumor-suppressor locus greatly increases the risk of developing certain forms of cancer. However, the majority of cancers are sporadic, and epidemiological data suggest that cancer-susceptibility in the general population is affected by multiple low-penetrance tumor-susceptibility genes [5,6].

Mouse models of cancer have been extensively used for analysis of the genetic basis of tumor-susceptibility, by which multiple cancer-susceptibility loci have been mapped [7,8]. Susceptibility for the two-stage skin carcinogenesis model varies among mouse strains [9,10], and a genetic approach has been used to identify the specific loci related to differences in tumor-susceptibility among the strains [4,7,8,11,12]. Fifteen skin tumor-susceptibility loci, *Skts1*~*15*, were mapped by analyzing an NIH and *Mus spretus* cross, in which *Mus spretus* is dominant-resistant against NIH [1,2]. A *Skts1* locus was also mapped by analyzing the Car-R and Car-S crosses [13], which were generated by the balanced intercross of eight inbred strains, including A/J, BALB/C, SJL/J, SWR/J, C57BL/6J, CBA/J, DBA/2 and P/J [9]. *Ps11*~*4* were mapped by using a C57BL/6J(B6) and DBA cross [14,15], and *Skts-fp1*~*3* were mapped by using a PWK and FVB cross [4]. Among them, only a limited number of corresponding genes for each locus has been identified. *Skts13* was identified as the *Aurka* gene [3], and *Skts14* as *Tgfb1* [16]. *Skts1* was suggested to be a tyrosinase gene [17]; however, none of the genes responsible for the remaining loci has yet been identified. The percentage of contribution of each tumor-susceptibility locus is relatively low, and usually each locus is mapped to a broad interval, which indicates multiple loci on the same chromosome and could be involved in tumor-susceptibility. Fine-resolution mapping of a tumor-susceptibility gene using conventional genetic analysis is a costly, time-consuming and labor-intensive process and, unfortunately, it often cannot pinpoint the locations of the tumor-susceptibility loci. The identification of *Aurka*, however, indicates the usefulness of a cross between heterogeneous stock and inbred animals for the conventional linkage in combination with haplotype mapping [3]. Wild-derived mouse strains, such as *Mus spretus* outbred, used for the *Skts13* identification, are generally derived from populations with much greater diversity than the classical strains and which have a more naturally arising pattern of variation. In addition, wild-derived strains can provide a glimpse into the geographical origins of genomic segments in the classical strains, which can be useful for haplotype-block analysis.

For this purpose, we analyzed a wild-derived *M. m. castaneus* outbred colony, which originated from Southeast Asian wild mice [18,19] and has been maintained for outbreeding, initially by Dr. Verne Chapman, then by Drs. Rosemary Elliott and Hiroki Nagase, for more than 20 years in the Roswell Park in-house facility. The colony has been maintained with 15~20 breeding pairs, and young animals from each breeder cages have been mated randomly avoiding crosses among siblings and sibling children. In this study, we found that *M. m. castaneus* is dominantly resistant to chemically induced skin tumors when crossed with a highly susceptible FVB strain [20]. Then we performed genetic analysis on a large backcross between FVB and *M. m. castaneus*. We compared the linkage pattern of each backcross progeny from different *M. m. castaneus* founders and demonstrated a distinct cancer-promotion phenotype, which may be explained by genetic heterogeneity within the *M. m. castaneus* colony.

Materials and Methods

Animals

Mice used in this study were bred in a specific pathogen-free (SPF) facility in the Department of Laboratory Animals at Roswell Park Cancer Institute and treated in accordance with Institutional Animal Care and Use Committee (IACUC) regulations. FVB/N mice were purchased from TACONIC (Germantown, NY). The *M. m. castaneus* outbred mice had been maintained at Roswell Park Cancer Institute for more than 20 years and had reached more than 65 generations when we started the present study. The *M. m. castaneus* mice were maintained avoiding inbreeding, even avoiding mating between first cousins. (FVB/N $\times$ *M. m. castaneus*) F1, abbreviated as FC, were generated from FVB/N and *M. m. castaneus* strains of mice. Sixteen FVB, 6 non-sibling *M. m. castaneus* at 66 generations and 20 FC male mice were subjected to the carcinogen treatment. These twenty FC mice were generated from 20 mating using independent 20 non-sibling *M. m. castaneus* males at 66–69 generations and 20 FVB/N females. Another set of five FC male mice (FC1, FC2, FC3, FC4 and FC5) were generated from five non-sibling *M. m. castaneus* males (founders 1, 2, 3, 4 and 5, respectively) at the 65 generations and five FVB/N females independently, and then they were backcrossed to FVB/N females again. Five pedigrees (pedigrees 1, 2, 3, 4 and 5) consisted of a total of 218 F1 backcross animals (FxFC) were generated from FC1, FC2, FC3, FC4 and FC5, respectively. Thirty-eight to 46 of the backcross mice were generated for each pedigree. Among them, the first 31 FxFC backcross mice from each of three FC founders, founders 1, 2 and 3, were picked up as the primary set of 93 FxFC backcross mice. In each cross, female parents are listed first. All of the animals used in this study are listed in supplemental table 1.

Skin Carcinogenesis

7.12-Dimethylbenz(a)anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) were purchased from Sigma-Aldrich Co. (St. Louis, MO). DMBA is used as a carcinogen and TPA as a promoter.

Six *M. m. castaneus* males, 20 FC male mice and 218 FxFC backcross male mice were treated with the two-stage skin carcinogenesis protocol.

The back-skin of each animal was carefully shaved with an electric shaver at ages 8–11 weeks. Two days after shaving, 200 μ l of DMBA (0.125 mg/ml solution in acetone) was applied to the back of each mouse. A total of 97.4 nmol of DMBA was applied to each mouse. One week after the treatment, the mice were treated with 400 μ l of TPA (5×10^{-5} M solution in acetone), i.e., 32.4 nmol of TPA, twice a week for 20 weeks. The animals were assessed twice a week for the appearance of papilloma. The number of papillomas was counted every other week until 20 weeks after the initial DMBA treatment. Papilloma incidence during the promotion phase, papilloma multiplicity and the size (average mm of 3 dimension diameters) of the papillomas 20 weeks after initiation were used to assess skin-tumor susceptibility. The size of the papillomas was able to analyze only in 180 tumor prone mice. The mice are carefully shaved once a month till the last day of papilloma counting.

Genotyping

Genotyping of the 26 *M. m. castaneus* and the 93 FxFC backcross mice was performed using an Illumina GoldenGate Assay (San Diego, CA). Genomic DNA was isolated using a QIAGEN DNeasy kit (Valencia, CA) from approximately 5mm of tail tissue from each mouse and then dissolved in distilled water. The quality and concentration of each DNA sample was assessed by measuring absorbance at OD 230nm, 260nm and 280nm using NanoDrop, then 2–3 μ g of each sample was applied to the analysis using an Illumina

GoldenGate assay mouse medium density linkage panel. Among 1,449 SNP loci, 522 SNPs were polymorphic between FVB and *M. m. castaneus* alleles of all 3 founders. From the 522 informative SNPs, 243 SNPs spreading at 9.9Mbp average intervals through all 19 autosomal chromosomes were chosen and used for the linkage analysis of the primary set with 93 FxFC backcrosses.

Genotyping of 218 FxFC backcross mice, including the primary set of 93 FxFC backcrosses, was performed using a total of 21 polymorphic microsatellite markers spread over Chromosome 4 with a 7.24 Mbp average interval. For this analysis, genomic DNA was isolated from mice-tail snips using the alkali method. Briefly, 2–3 mm of the tails were boiled in 300 μ l of 50 mM NaOH for 30 minutes, then 25 μ l of 1 M Tris-HCL (pH 8.0) was added. The DNA was genotyped using the 21 microsatellite markers by polymerase chain reaction (PCR) and then the reaction products were separated by gel electrophoresis using 1% agarose or 3% Nusieve-GTG low-melting-temperature agarose gels in a 0.5 $\times$ TBE buffer.

For the haplotype analysis, tail genomic DNA of five FC founders were genotyped with 169 microsatellite markers spread over Chromosome 4 with a 0.9 Mbp average interval. Three SNP markers were used to confirm the position of the haplotype block commonly observed in FC1 and FC2 but not in FC3, FC4 and FC5.

Statistical analysis

J/ql, a Java GUI (Graphical User Interface) for the popular Quantitative trait loci (QTL) data-analysis software R/ql [21], was used to analyze phenotype and genotype data from the FxFC F1 backcross to map QTLs related to susceptibility for chemically induced skin tumors. A permutation test was performed with 10,000 permutations to estimate the empirical threshold value for the mapping. Interval mapping was performed to find the linkage for all autosomal chromosomes.

Results

Using the two-stage skin tumor induction protocol, all seven FVB mice developed papillomas with an average multiplicity of 38.14 ± 5.36 by 20 weeks after initiation. However, none of the six *M. m. castaneus* mice developed any papillomas on the dorsal skin (Table 1). Five out of the 20 FC mice developed one or two tumors; however, the average multiplicity was 0.3 ± 0.12 (Table 1). The differences in papilloma multiplicity between FVB and *M. m. castaneus* and between FC and FVB were significant ($p < 0.001$). These results demonstrate that the *M. m. castaneus* strain is a dominant-resistant phenotype to DMBA/TPA-induced skin tumors against a susceptible FVB strain.

Then, 218 F1 backcross FxFC progenies were generated from five independent FC founders. Since *M. m. castaneus* is maintained within a closed colony, mating to avoid inbreeding, animals were supposed to have genetic heterogeneity. An analysis of 26 individuals at the 68th generation using Illumina GoldenGate SNPs array with 1,449 probes showed that 139 loci (10.3%) out of 1,345 successfully genotyped SNP loci were polymorphic among animals in the colony (Supplemental Figure 1). Five FC mice were generated from five non-sibling *M. m. castaneus* males and five FVB/N females independently, then backcrossed to FVB/N again to generate 218 FxFC.

A preliminary linkage analysis was performed by genotyping 93 FxFC backcross mice derived from three FC founders for 243 informative SNP markers spread throughout the genome. The number and average size of papillomas 20 weeks after the initial treatment were used as traits. Seventy-four mice (79.56%) developed papillomas, and the average

multiplicity was 9.37 ± 1.07 . The average papilloma size among the 74 mice that developed papillomas was 2.09 ± 0.12 mm (Table 2). Genome-wide significant linkage for tumor multiplicity ($P < 0.05$) was found only on Chromosome 4. A genome-wide suggestive linkage ($P < 0.63$) was also found on chromosomes 3 and 11 (Fig. 1A). No significant linkage was observed for average papilloma size; however, suggestive linkages ($P < 0.63$) were mapped on chromosomes 4, 5 and 8 (Fig. 1B).

Further analysis was then conducted with 218 FxFC backcross mice, which included the 93 FxFC backcrosses from the preliminary linkage analysis. One hundred eighty mice (82.56%) developed papillomas, and the average multiplicity was 10.07 ± 0.78 (Table 2). Average papilloma size among the 180 tumor prone mice was 2.32 ± 0.1 mm. Interval mapping was performed with 19 informative microsatellite markers on Chromosome 4, and significant linkage ($P < 0.05$) for the papilloma multiplicity was mapped between D4Mit97 (29.3Mbp) and D4Mit311 (141.6 Mbp) (Fig. 2). A maximum LOD score of 14.4 was mapped at the D4Mit26 locus (87.3Mbp).

In addition to a large backcross analysis using 218 mice, each family generated from the five independent heterogeneous *M. m. castaneus* founders was also analyzed independently. Among the five pedigrees, the incidence of papillomas, papilloma multiplicity and the size of the papillomas varied from 67.39% to 95.65%, from 8.43 ± 1.25 to 12.75 ± 1.75 and from 2.05 ± 0.21 mm to 2.93 ± 0.34 mm, respectively. These differences were not statistically significant (Table 2). A significant linkage for papilloma multiplicity was detected at the D4Mit26 (88.8 Mbp) locus on Chromosome 4 in pedigrees 1, 2, 3 and 5 and suggestive linkage in pedigree 4 (Table 3-1). On the other hand, a significant linkage on chromosome 4 was mapped in two pedigrees from FC1 and FC2, while no linkage was observed in pedigrees 3, 4 and 5, when papilloma size was used as a quantitative trait (Table 3-2, Fig. 3). Genotypes at D4Mit289 were significantly associated with papilloma size in both pedigrees 1 and 2 (Table 3-2). A significant linkage was mapped between D4Mit91 (43.8Mbp) and D4Mit165 (90.7 Mbp) on chromosome 4 (Fig. 3), with the highest LOD score, 6.1, at marker D4Mit289 (81.1 Mbp) when pedigrees 1 and 2 were analyzed together. The evidence of linkage is, however, not observed in the pedigrees from FC3, FC4 and FC5 (Table 3-2, Fig. 3).

Linkage disequilibrium (LD) mapping of the five FC founders was performed to find FC1 and FC2's specific haplotype block using 169 microsatellite markers spanning at 0.90 Mbp average intervals through Chromosome 4. In this provisional analysis, only D4Mit345 (21.6 bp) showed a common genotype between founders 1 and 2. An estimated maximum span of the common haplotype block was between D4Mit293 (20.7 Mbp) and D4Mit171 (22.4 Mbp) (Supplemental Table 2 and Figure 4). D4Mit345 and papilloma size were associated in FC1 and FC2, but not in FC3, FC4 and FC5 (Supplemental Figure 2). Genotyping of an additional three SNP markers, Gm11875, Sfrs18 and Fbx114, which is located within this interval, also segregated FC 1 and 2 from FCs 3, 4 and 5.

Since suggestive linkages for papilloma size were identified at loci on chromosomes 5 and 8 using 74 informative mice by the preliminary linkage analysis (Fig. 1B), the association between markers located near the highest peak and the number of papillomas was also analyzed for 165 informative animals, including the 74 mice. By marker-regression analysis, suggestive linkages ($P < 0.63$) were found when all families were taken together, but none of them reached a suggestive linkage level when each family was analyzed independently. (data not shown).

Discussion

The present study indicates that *M. m. castaneus* is a dominant-resistant strain to two-stage skin carcinogenesis when it is crossed with highly susceptible FVB mice. No *M. m. castaneus* developed papillomas. Five out of 20 FC F1 hybrid mice developed papillomas; however, all of the five had only one or two papillomas. In a study of 218 FVB and *M. m. castaneus* backcross mice, a significant QTL with a maximum LOD score of 14.4 was mapped on Chromosome 4, and that was overlapped with *Skts-fp1*, which has been mapped by analysis of the FVB and PWK cross [4].

The maximum linkage for papilloma multiplicity was observed between D4Mit289 (81.1Mbp) and D4Mit165 (90.7Mbp) and was overlapped with one of the two highest peaks of *Skts-fp1*, located between D4Mit289 (81.1Mbp) and D4Mit153 (96.1Mbp), which was mapped in the FVB and PWK cross [4]. Another peak of around D4Mit146 (109.4 Mbp) in that cross was however, not observed in the linkage analysis of the FVB and *M. m. castaneus* backcross. A common skin cancer-susceptibility gene might be identified or narrowed down to a small interval where it was localized between D4Mit289 (81.1Mbp) and D4Mit153 (96.1Mbp) by a comparative analysis between *M. m. castaneus* and PWK.

Considering the genetic heterogeneity in a heterogeneous outbred stock, haplotype analysis should be applied in conjunction with backcross analysis. When each pedigree was analyzed separately depending on each founder, the overall linkage pattern for papilloma multiplicity was similar in each family (Table 3-1, Fig. 2); however, there were some discrepancies among pedigrees when a QTL analysis for papilloma size was performed (Table 3-2, Fig. 3) on Chromosome 4. These results suggest that there are at least two genetic factors located in the single highest broad linkage-peak on Chromosome 4. A papilloma-multiplicity phenotype could be associated with both susceptibility at the initiation and promotion stage, but perhaps mainly associated with the susceptibility at the initiation stages, while papilloma size may indicate susceptibility mainly at the promotion stage. Thus, a broad QTL peak centered over D4Mit26 for papilloma multiplicity may be related mainly to tumor initiation but a little to the promotion stage, while QTL near D4Mit289 for papilloma size may be involved in susceptibility mainly at promotion stages.

LD mapping of F1 founders revealed that there could be at least one haplotype block corresponding to three polymorphic markers between D4Mit293 (20.7 Mbp) and D4Mit171 (22.4 Mbp), which showed a common LD pattern segregating FC1 and FC2 versus FC3, 4 and 5 (Fig. 4). None of the other markers on Chromosome 4 showed the same haplotype pattern as those seen at this region. It is possible that the genetic factor located on this haplotype block affects the papilloma size. Nine known genes and six or seven predicted genes have been mapped within the LD block (Supplemental Table 3). No common haplotype was found among FC1, FC2 and known resistant strains of PWK and *Mus spretus* within this interval (Data not shown). Detailed analysis of the haplotype block between D4Mit293 and D4Mit171 is ongoing to define the exact boundary of the haplotype block and find a corresponding genetic factor. However, the fact is that this haplotype block is located relatively far from the highest peak of the FC 1 and FC2 specific QTL for papilloma size, which was mapped around D4Mit289 (81.1 Mbp). Even though the FC 1 and FC2-specific peak is located around 81.1 Mbp, there was no variation among the five founders between D4Mit274 (54.5 Mbp) and D4Mit175 (100.1 Mbp), and founders 1, 2 and 3 share a common genotype from D4Mit274 (54.5 Mbp) to D4Mit51 (154.4 Mbp), except for markers D4Mit75 (104.6 Mbp). It is possible that the haplotype block between D4Mit293 and D4Mit171 has a trans effect on the linkage peak of D4Mit289. Further analysis is needed to prove that this haplotype block is a corresponding region for the papilloma size QTL.

Other suggestive linkages for papilloma size were mapped on chromosomes 5 and 8, but a similar genetic difference among families was not observed on these chromosomes, suggesting that familial difference in QTL on Chromosome 4 is not affected by the suggestive linkage on chromosomes 5 and 8.

One of the problems with genetic analysis of complex traits, including tumor-susceptibility, is the difficulty in mapping the QTL in a very small interval. In conventional F1 backcross studies using inbred strains, the significant linkage of each QTL generally spans a 20–40cM region, which contains hundreds of genes. To solve this low-resolution problem, several new genetic-mapping strategies such as recombinant congenics [22], advanced intercross lines [23] and Collaborative Cross [24], have been developed. Accumulated haplotype knowledge about mouse inbred strains enables us to do multiple mapping crosses or *in silico* mapping [25–28]. Whole Genome Association Study allows investigators to narrow down the lung cancer-susceptibility loci, *Pas1*, to within a 0.5Mbp interval [29]. Heterogeneous stocks have been established using inbred strains, and multiple QTLs are mapped with an average 95% confidence interval of 2.8Mbp [30]. Very recently, a *spretus* and FVB backcross analysis using a whole-genome expression pattern (eQTLs) of tail RNAs from each backcross animal allowed the description of the genetic architectures of skin carcinogenesis [31]. All of the approaches could help us to understand the genetic basis of mouse complex genetic trait and the identification of genetic components responsible for the complex genetic trait of cancer susceptibility.

Outbred stocks are also known to be a very useful tool for the genetic analysis of complex traits [32,33]. The outbred stocks accumulate many breakpoints that split chromosomes into small haplotype blocks, enabling us to perform high-resolution mapping of QTLs. *Aurka* was identified as the *Skts13* corresponding gene [3] by analyzing haplotype segregation among outbred and inbred *Mus spretus* stocks. The *Rgs2* gene was identified as a corresponding gene for mouse anxiety-related QTL by genetic analysis of the MF1 outbred stock [34]. In the present study, the wild-derived *M. m. castaneus* outbred colony in our laboratory was shown to have 10.3% genetic heterogeneity. The average SNP variance among 47 experimental mouse inbred strains was 33.7% when they were assessed by an Affymetrix Mouse Mapping 5K SNP kit [35]. There was 16.1% SNP variance among C57 sub-strains and 3.8% among 129 sub-strains in the same analysis. In addition, our *M. m. castaneus* outbred colony demonstrated its phenotypic heterogeneity of behavioral phenotype (personal communication with Dr. Joseph Takahashi and Dr. Kazuhiro Shimomura in Univ. Texas Southwestern). According to these facts, analysis using this outbred colony could enable us to identify a genetic factor for remaining tumor-susceptibility loci.

M. m. castaneus is a wild-derived strain, and high-resolution analysis of mouse strains with 8.27 million SNPs showed that *M. m. castaneus* provides only 3% of the genetic contribution for the classical inbred strain, including FVB [36]. High genetic and phenotypic diversity, however, did not result in finding all the multiple genetic components involved in skin tumor-susceptibility, as seen in the NIH and *mus spretus* cross. In the analysis of the FVB and PWK cross, a significant linkage to skin papilloma number was mapped, again only on Chromosome 4. However, further analysis using a phenotype-driven congenic study has revealed that many other small QTLs other than *Skts-fp1* contributed to keeping animals in a completely resistant phenotype for skin carcinogenesis (Fujiwara et al. unpublished). In the present analysis of the FVB and *M. m. castaneus* cross, we mapped evidence of linkages on chromosomes 3, 5, 8 and 12, each of which contributed some degree of phenotypic variation and may have the presence of functionally polymorphic haplotype blocks within the interval. Further analysis using an increased number of mice may allow us to find low-penetrance cancer-susceptibility QTLs for skin tumorigenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DMBA	7,12-dimethylbenz(a)anthracene
TPA	12-O-tetradecanoylphorbol-13-acetate
<i>M. m. castaneus</i>	<i>Mus musculus castaneus</i>
FC	FVB × <i>M. m. castaneus</i>
FxFC	FVB × (FVB × <i>M. m. castaneus</i>)
PCR	polymerase chain reaction
TBE	tris boric EDTA buffer
SNP	single nucleotide polymorphism
JAVA GUI	Java Graphical User Interface
LD	Linkage disequilibrium
WGAS	Whole Genome Association Study

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Figure1 A

Figure1 B

Figure 1.

Whole-genome scan of 93 FxFC backcross mice. (A) Interval mapping revealed highly significant linkage on chromosome 4 and suggestive linkage on chromosomes 3 and 12 for papilloma multiplicity 20 weeks after initiation. Dashed line indicates empirical suggestive ($P<0.63$), significant ($P<0.05$) and highly significant ($P<0.01$) linkages of LOD score at 1.6, 3 and 3.5, respectively. (B) Interval mapping revealed suggestive linkage on chromosomes 4, 5 and 8 for average papilloma size 20 weeks after initiation. Dashed line indicates empirical suggestive linkage ($P<0.63$) of LOD score at 1.6.

Figure 2.

Interval mapping for papilloma multiplicity QTL on chromosome 4. By analyzing 218 FxFC backcross mice, the highly significant linkage for papilloma multiplicity 20 weeks after initiation was mapped on chromosome 4 (black line). Dark gray indicates the linkage calculated for founders 1 and 2, right; gray is for founders 3, 4 and 5. Physical position (Mbp) of each marker is shown on the bottom.

Figure 3.

Interval mapping for papilloma size QTL on chromosome 4. By analyzing 218 FxFC backcross mice, the highly significant linkage for the average papilloma size 20 weeks after initiation was mapped on chromosome 4 (black line). Dark gray indicates the linkage calculated for founders 1 and 2, light gray is for founders 3, 4 and 5. Physical position (Mbp) of each marker is shown on the bottom.

Marker	Location (Mbp)	FC1	FC2	FC3	FC4	FC5	FVB
D4Mit260	18.95	2	2	2	2	2	1
D4Mit135	19.79	1	2	3	3	3	1
D4Mit293	20.66	2	3	2	2	2	1
Gm11875	21.55	1	1	2	2	2	1
D4Mit345	21.66	2	2	3	3	3	1
Sfrs18	21.77	1	1	2	2	2	1
Fbxl4	22.28	2	2	1	1	1	1
D4Mit171	22.41	2	3	2	2	2	1
D4Mit96	23.55	2	2	2	2	2	1
D4Mit211	24.27	2	2	2	2	2	1

Figure 4.

Haplotype map of FC founders on whole Chromosome 4. (A) Five FC founders and FVB were genotyped using 169 microsatellite markers. Genotype data of Castaneus allele in each FC founder was shown as polymorphic allele ID. (B) Region between D4Mit293 and D4Mit171 is genotyped using additional SNP markers.

Table 1

Incidence and multiplicity of papilloma in the parental strains and F1

Strain	No. of animals	Incidence (%)	Multiplicity ^{a)}
FVB/N	7	7 (100)	38.14 ± 5.36
Castaneus	6	0 (0)	0
F × C	20	5 (25.0)	0.3 ± 0.12

^{a)} data are shown as mean ± SEM.

Table 2

Incidence, multiplicity and size of papilloma in backcross

Founders	No. of animals	Incidence (%)	Multiplicity ^{a)}	Size of papilloma (mm) ^{b)}
Small set	93 ^{c)}	74(79.56)	9.37 ± 1.07	2.09 ± 0.12
Total set	218	180(82.56)	10.07 ± 0.78	2.32 ± 0.10
Founder1	46	31(67.39)	10.19 ± 1.84	2.21 ± 0.23
Founder2	44	41(93.18)	12.75 ± 1.75	2.39 ± 0.24
Founder3	38	32(84.21)	9.63 ± 1.76	2.18 ± 0.20
Founder4	44	32(72.72)	9.36 ± 1.98	2.93 ± 0.34
Founder5	46	44(95.65)	8.43 ± 1.25	2.05 ± 0.21

^{a) b)} data are shown as mean ± SEM.

^{c)} Including each 31 FxFC backcross from founder 1,2 and 3.

Table 3

Association between tumor number and D4Mit26

Founders	No. of animals	Average number of papillomas		LOD score ^{b)}
		FF ^{a)}	FC ^{a)}	
ALL	216	14.79 ± 1.14 (118)	4.42 ± 0.69 (98)	14.4 ^{***}
Founder1	46	17.55 ± 2.99 (20)	4.54 ± 1.64 (20)	3.3 ^{**}
Founder2	43	16.83 ± 2.21 (29)	4.86 ± 1.54 (14)	3.5 ^{**}
Founder3	37	14.11 ± 2.41 (19)	4.72 ± 2.26 (18)	3.0 ^{**}
Founder4	44	12.81 ± 2.95 (27)	3.88 ± 1.35 (17)	1.8 [*]
Founder5	46	12.70 ± 2.04 (23)	4.17 ± 0.81 (23)	3.4 ^{**}

Association between tumor size and D4Mit289

Founders	No. of animals	Average size of papillomas (mm)		LOD score ^{b)}
		FF ^{a)}	FC ^{a)}	
ALL	169	2.70 ± 0.14 (101)	1.75 ± 0.15 (68)	6.2 ^{***}
Founder1	32	2.94 ± 0.35 (17)	1.39 ± 0.11 (15)	3.0 ^{**}
Founder2	36	3.01 ± 0.28 (24)	1.25 ± 0.16 (12)	3.3 ^{**}
Founder3	30	2.13 ± 0.25 (16)	2.11 ± 0.33 (14)	0.0
Founder4	28	3.25 ± 0.40 (19)	2.26 ± 0.59 (9)	0.5
Founder5	43	2.21 ± 0.23 (25)	1.84 ± 0.39 (18)	0.4

^{a)}Data are shown as mean ± SEM.

Values within the parenthesis are number of animals.

^{b)}LOD score was calculated by interval mapping.

* suggestive

** significant

*** highly significant