An Imprinted Locus Epistatically Influences *Nstr1* and *Nstr2* to Control Resistance to Nerve Sheath Tumors in a Neurofibromatosis Type 1 Mouse Model

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Abstract

Cancer is a complex disease in which cells acquire many genetic and epigenetic alterations. We have examined how three types of alterations, mutations in tumor suppressor genes, changes in an imprinted locus, and polymorphic loci, interact to affect tumor susceptibility in a mouse model of neurofibromatosis type 1 (NF1). Mutations in tumor suppressor genes such as *TP53* and in oncogenes such as *KRAS* have major effects on tumorigenesis due to the central roles of these genes in cell proliferation and cell survival. Imprinted genes expressed from only one parental chromosome affect tumorigenesis if their monoallelic expression is lost or duplicated. Because imprinted loci are within regions deleted or amplified in cancer, the parental origin of genomic rearrangements could affect tumorigenesis. Gene polymorphisms can vary tumor incidence by affecting rate-limiting steps in tumorigenesis within tumor cells or surrounding stroma. In our mouse model of NF1, the incidence of tumors mutant for the tumor suppressor genes *Nf1* and *Trp53* is strongly modified by a linked imprinted locus acting epistatically on two unlinked polymorphic loci, *Nstr1* and *Nstr2*. This interaction of an imprinted locus and polymorphic susceptibility loci has profound implications for human mapping studies where the parental contribution of alleles is often unknown. (Cancer Res 2006; 66(1): 62-8)

Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease affecting 1 in 3,500 people and is caused by mutations in the *NF1* gene (*Nf1* in the mouse; ref. 1). A study comparing monozygotic twins and more distant relatives has shown that polymorphic loci unlinked to *NF1* alter the severity of the disease (2) although these loci have not yet been identified. NF1 is characterized by benign tumors of the central and peripheral nervous systems, such as optic pathway gliomas and neurofibromas, respectively. In addition to benign tumors, NF1 patients have an increased risk for developing particular malignancies, including malignant peripheral nerve sheath tumors (MPNST) and malignant astrocytomas including glioblastoma (1). MPNSTs arise in the nerves of NF1 patients and have been shown to originate from Schwann cells (3, 4) often through malignant transformation of benign plexiform neurofibromas. As part of this malignant transformation from plexiform neurofibroma to MPNST, the p53 tumor suppressor (encoded by the *Tp53* gene in humans and the *Trp53* gene in mice) has been shown to be mutated or lost (5). Because these tumors are highly aggressive and currently incurable, a greater understanding of how they arise may lead to novel treatments. Because MPNSTs are currently incurable and evidence from humans suggests that modifier genes affect the severity of NF1 (2, 6), we have used a mouse model of the malignancies associated with NF1 (7–9) to study the role of different genetic factors that influence MPNST tumorigenesis.

We and others have developed a mouse model to study NF1 by combining mutations in the murine *Nf1* gene and *Trp53* gene together on the same chromosome (*NPcis* mice; refs. 7–10). The product of the *Nf1* gene, neurofibromin, is a rasGAP protein involved in the down-regulation of ras signaling. Because the *Nf1* and *Trp53* genes are closely linked on mouse chromosome 11, loss of the wild-type (wt) copies of the genes in this model occurs as a single genomic event, subsequently leading to concurrent up-regulation of ras signaling and loss of p53. These mice develop many of the malignancies associated with NF1 in patients, including aggressive peripheral nerve sheath tumors [genetically engineered murine (GEM) PNSTs; refs. 7, 9, 11] as well as malignant astrocytoma and glioblastoma (8, 10). Because these mice are on genetically well-defined strain backgrounds and the incidence of tumors is completely penetrant by 1 year of age, we are using these mice to investigate the genetic and epigenetic effects on tumor susceptibility. Candidate modifiers of tumor susceptibility can then be tested for their role in the variable expressivity of NF1.

There has been a growing appreciation over the past 20 years of the role of epigenetic changes in tumorigenesis (12). Much of the data have pointed to the role of methylation in the silencing of tumor suppressor genes or in the deregulation of imprinted gene expression as a step in tumorigenesis. In addition to epigenetic alterations, expression of imprinted genes may be altered by genomic changes such as the loss or reduplication of chromosomes. We have recently shown that an imprinted locus linked to

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Nf1 and *Trp53* on mouse chromosome 11 affects susceptibility to astrocytoma depending on whether tumors lose the maternal or paternal copy of mouse chromosome 11 (10). This work has suggested that linkage of an imprinted locus to a tumor suppressor gene can influence the rate of tumorigenesis (for further discussion, see ref. 13).

In addition to interactions between imprinted loci and major tumor suppressor genes, such as *Trp53*, we wanted to test whether there were interactions between imprinted loci and low-penetrance polymorphic modifier genes. Modifier genes can alter the phenotype of mutations in tumor suppressor genes (or oncogenes) depending on the genetic background of the individual (14). Unlike the highly penetrant mutations in tumor suppressor genes, polymorphic modifier genes exert a more subtle effect through their normal functions by changing the efficiency of different steps in tumorigenesis. These steps could occur within the tumor cell, such as the efficiency of cell cycle checkpoints or DNA repair, or systemically in the organism, such as the efficiency of antitumor immune responses. We have further explored the role of the imprinted locus in the context of polymorphic modifier genes in the *NPcis* mouse model by focusing on GEM PNSTs. In this mouse model, GEM PNSTs arise at younger ages than astrocytomas and are easier to identify and to score as a binary trait, allowing us to map modifiers more quickly. Similar to human MPNSTs, the GEM PNSTs in *NPcis* mice show mitotic figures, spindle cells, nerves within the tumor, and are variably positive for the tumor markers S100, p75 (low-affinity nerve growth factor receptor), desmin, and collagen IV (Fig. 1; ref. 15). The majority of these tumors are negative for the rhabdomyosarcoma markers Myf4 and MyoD1 (data not shown; ref. 16) although we occasionally see tumors with regions of Myf4 and MyoD1 immunoreactivity (4 of 20 examined). This suggests that these tumors may also include malignant Triton tumors, a variant of MPNSTs seen in NF1 patients.

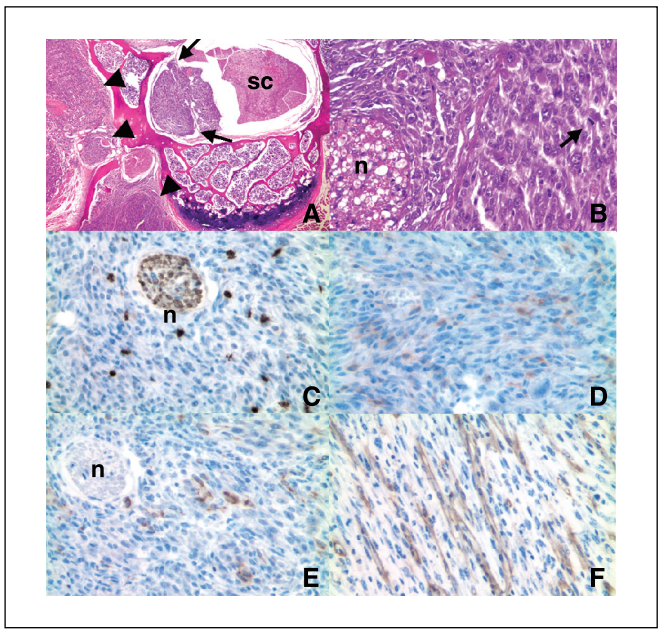

Figure 1. *NPcis* mice develop GEM PNSTs with characteristics of human MPNSTs. *A,* low-magnification view of a GEM PNST forming along a nerve root ganglion of the lower spinal cord (*sc*). *Arrows,* tumor in the ganglion; *arrowheads,* tumor invasion into surrounding tissue along the spine. *B,* high-magnification view of the tumor from (*A*) with a nerve (*n*) visible within the tumor and a mitotic figure (*arrow*). *C,* positive immunoreactivity in a GEM PNST for S100 including a nerve as a positive control for staining. *D,* positive immunoreactivity for p75. *E,* rare positive immunoreactivity for desmin. *F,* positive immunoreactivity for the basement membrane marker collagen IV.

Table 1. Frequency of GEM PNST in *NPcis* mice

Progeny	Total	With GEM PNST (%)
NPcis, B6	**138**	**77 (56%)**
NPcis^{mat}, B6*	47	18 (38%)
NPcis^{pat}, B6†	91	59 (65%)
NPcis, B6 × A	**100**	**29 (29%)**
NPcis^{mat}, B6 × A*	80	20 (25%)
NPcis^{pat}, A × B6†	20	9 (45%)
NPcis, B6 × A × B6	**254**	**131 (52%)**
NPcis^{mat}, (B6 × A) × B6*	110	42 (38%)
NPcis^{pat}, B6 × (A × B6)†	144	89 (62%)

*$NPcis^{mat}$ mice inherit the *NPcis* mutant chromosome from their mother.
†$NPcis^{pat}$ mice inherit the *NPcis* mutant chromosome from their father.

Materials and Methods

Generation of *NPcis* mice on different genetic backgrounds. We bred *NPcis* mice at Massachusetts Institute of Technology and National Cancer Institute (NCI)-Frederick as previously described (10) according to the guidelines and regulations of the Animal Care and Use Committee at each institution. We compared cohorts of C57BL/6J-*NPcis* and C57BL/6J × A/J-*NPcis* bred at Massachusetts Institute of Technology and NCI to determine if the phenotype was reproducible and found no significant differences between the different cohorts. The results shown in Table 1 and Fig. 2 are pooled from the two institutions. We genotyped the *NPcis* mice by PCR as previously described (10) and aged the mice until tumors developed as described previously (8).

Pathology and immunohistochemistry of GEM PNSTs. H&E-stained sections were scored for the presence of spindle cell sarcomas by K.M.R. and R.T.B. A subset of the sarcomas (n = 20) was characterized with antibodies. We stained sections of tumors fixed in 10% formalin and paraffin embedded with 1:1,000 anti-S100 (DakoCytomation, Carpinteria, CA), 1:100 anti-p75 (Chemicon, Temecula, CA), 1:100 anti-desmin (MP Biomedical/Cappel, Irvine, CA), 1:50 anti-collagen IV (MP Biomedical/Cappel), 1:30 anti-Myf4 (clone L026, Novocastra, Newcastle upon Tyne, United Kingdom), and 1:50 anti-MyoD1 (clone 5.8A, DakoCytomation). Slides to be stained with anti–collagen IV were predigested with pepsin. All antibodies were detected using biotinylated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA), the Vector Elite ABC kit, and 3,3′-diaminobenzidine substrate for peroxidase (Vector Laboratories, Burlingame, CA). For phenotyping of backcross progeny for genome-wide mapping of modifier loci, mice were considered to have a GEM PNST based on the characteristic spindle cell morphology of these tumors in H&E-stained sections. All backcross progeny were scored by K.M.R. for consistency.

Genome-wide simple sequence length polymorphism genotyping. To map modifiers in (C57BL/6J × A/J) × C57BL/6J and C57BL/6J × (A/J × C57BL/6J) backcross progeny, we genotyped tail DNA at simple sequence length polymorphism markers (ResGen MapPairs, Invitrogen, Carlsbad, CA) listed in Supplementary Table S1 and ran them on 3% Metaphor agarose (Cambrex, East Rutherford, NJ) gels in 1× Tris-borate EDTA buffer.

Linkage analysis and statistical methods. We did multipoint, binary trait linkage analysis (17, 18) separately in individuals who received the mutation from their father and mother with the genetic mapping software R/qtl (19), an add-on package to the general statistical software R (20). We used a permutation test (21) to establish statistical significance. *P* values adjusted for the genome-wide scans were estimated as the proportion of permutation replicates in which the maximum LOD score, genome-wide, was greater than or equal to the observed LOD score.

Assessing allelic imbalance by single-nucleotide polymorphisms and simple sequence length polymorphisms. We designed TaqMan PCR assays for single-nucleotide polymorphisms using Assay-by-Design[6] or Primer-Express software (Applied Biosystems, Inc., Foster City, CA; Supplementary Table S2). The ratio of C57BL/6J to A/J DNA in the tumor was determined by normalizing the samples to a standard curve of multiple ratios of C57BL/6J genomic DNA to A/J genomic DNA (The Jackson Laboratory, Bar Harbor, ME) using SDS2.1 (Applied Biosystems). We ran simple sequence length polymorphism markers on chromosomes 15 and 19 on tumor DNA as described above. Densitometry on the ethidium-stained bands was done using the AlphaEase FC software (version 4.0.1) on the AlphaInnotech ChemiImager 5500. Between 2 and 5 replicates were analyzed for each tumor sample and between 5 and 14 C57BL/6J × A/J F1 controls were analyzed to determine the reproducibility of the assays. Samples were determined to have allelic imbalance if they showed >30% change in C57BL/6J from the average C57BL/6J value in F1 controls and if the C57BL/6J value of the tumor DNA fell outside the 95% prediction interval (22) of the F1 mean.

Generation of the haplotype map. SNPview[7] was used to search for haplotype blocks, ordered according to Public Assembly v.3a. Single-nucleotide polymorphism alleles C57BL/6J, C3H/HeJ, DBA/2J, A/J, 129S1/SvImJ, and CAST/EiJ and simple sequence length polymorphism alleles C57BL/6J and A/J were used to build the haplotype blocks. The region between 31.52 and 52.07 Mb on chromosome 15 and the region between 0 and 6.25 Mb on chromosome 19 were examined.

Determining syntenic regions in human MPNSTs. By comparing Mouse Genome Build 34 and Human Genome Build 35.1, the candidate regions were matched to human chromosomal regions using Comparative Maps.[8]

Results

We have examined how polymorphisms in the strain background affect GEM PNSTs by crossing *NPcis* mice on the C57BL/6J background (C57BL/6J-*NPcis*) to several different strain backgrounds. We have previously shown that strain background strongly affects the tumor spectrum in these mice, with C57BL/6J-*NPcis* mice being more susceptible to astrocytomas than *NPcis* mice on a 129S4/SvJae background (129S4/SvJae-*NPcis*; ref. 10). C57BL/6J-*NPcis* are also highly susceptible to GEM PNSTs (Table 1; Fig. 2). Whereas the 129S4/SvJae strain and the CBA/J strain are resistant to astrocytoma (10), we found no effect of these backgrounds on GEM PNST incidence (Fig. 2). Conversely, the A/J strain caused *NPcis* mice to be more resistant to GEM PNSTs in C57BL/6J × A/J F1 crosses (P = 0.0004, χ^2 test; Table 1). We also found a marked reduction in GEM PNSTs in *NPcis* mice on DBA/2J × C57BL/6J F1 and CAST/EiJ × C57BL/6J F1 backgrounds (data not shown). We did not note any significant change in the age of onset of the GEM PNSTs on different strain backgrounds or in reciprocal crosses. The effect of strain background on astrocytoma and GEM PNST incidence suggests that polymorphic modifier genes act in tissue-specific ways. Of the seven strains compared with C57BL/6J, none showed an overall reduction in tumorigenesis that might suggest a global modification of *Nf1* or *Trp53*. This is consistent with the idea that polymorphic modifier genes act on the context in which the cancer develops and on cell type–specific functions of *Nf1* and/or *Trp53*.

In our previous study on astrocytoma susceptibility in *NPcis* mice, we showed that inheritance of *NPcis* mutant chromosome 11 from the mother increased susceptibility to astrocytoma (10). We therefore tested whether the inheritance of the *NPcis* mutant chromosome from the mother versus the father made a difference in GEM PNST incidence. We crossed C57BL/6J-*NPcis* fathers to wt C57BL/6J mothers and selected C57BL/6J-*NPcis* progeny for analysis. We similarly crossed C57BL/6J-*NPcis* mothers to wt C57BL/6J fathers and selected C57BL/6J-*NPcis* progeny for analysis. We aged the progeny of these two groups until tumors formed and compared the incidence of GEM PNSTs in the two populations (Table 1). Whereas the progeny of *NPcis* mothers (*NPcis*^{mat}) are more susceptible to astrocytoma, we found that the progeny of *NPcis* fathers (*NPcis*^{pat}) are more susceptible to GEM PNSTs (Table 1). This effect on susceptibility is independent of strain background and can be seen within the C57BL/6J-*NPcis* strain (P = 0.003, χ^2 test).

We also observe increased susceptibility to GEM PNSTs in *NPcis*^{pat} progeny when the strain background is varied. When C57BL/6J-*NPcis* fathers are crossed to wt A/J mothers, the *NPcis*^{pat} progeny are more susceptible to GEM PNST than the *NPcis*^{mat} progeny of C57BL/6J-*NPcis* mothers crossed to wt A/J fathers (P = 0.08, χ^2 test; Table 1). As a final test, we crossed

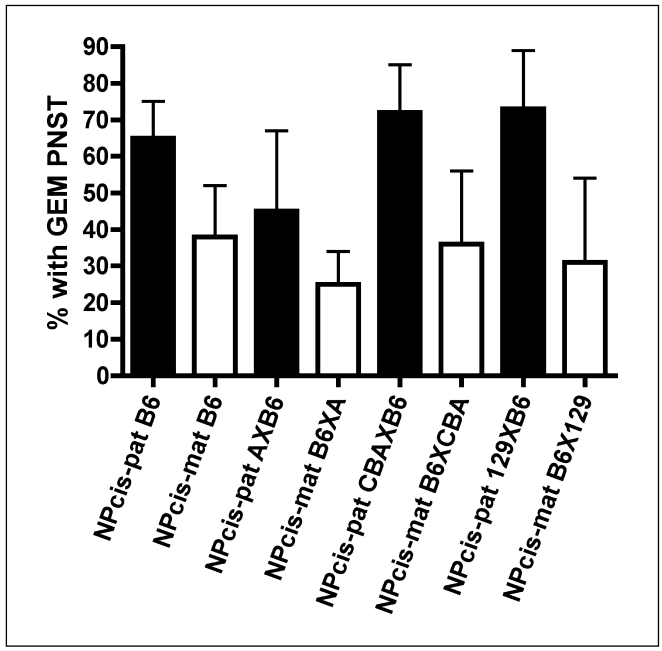

Figure 2. Frequency of GEM PNSTs on different F1 strain backgrounds compared with C57BL/6J-*NPcis* (*B6*). *Black columns,* incidence of GEM PNSTs in F1 progeny of C57BL/6J-*NPcis* fathers crossed to different wt mothers; *white columns,* incidence in F1 progeny of C57BL/6J-*NPcis* mothers crossed to different wt fathers. *Bars,* 95% confidence interval. C57BL/6J-*NPcis*^{pat} (*NPcis-pat B6*), n = 91; C57BL/6J-*NPcis*^{mat} (*NPcis-mat B6*), n = 47; A/J × C57BL/6J-*NPcis*^{pat} (*NPcis-pat A×B6*), n = 20; C57BL/6J × A/J-*NPcis*^{mat} (*NPcis-mat B6×A*), n = 80; CBA/J × C57BL/6J-*NPcis*^{pat} (*NPcis-pat CBA×B6*), n = 46; C57BL/6J × CBA/J-*NPcis*^{mat} (*NPcis-mat B6×CBA*), n = 22; 129S4/SvJae × C57BL/6J-*NPcis*^{pat} (*NPcis-pat 129×B6*), n = 30; C57BL/6J × 129S4/SvJae-*NPcis*^{mat} (*NPcis-mat B6×129*), n = 16.

[6] http://www.appliedbiosystems.com.
[7] http://snp.gnf.org/.
[8] http://www.ncbi.nlm.nih.gov/Homology/.

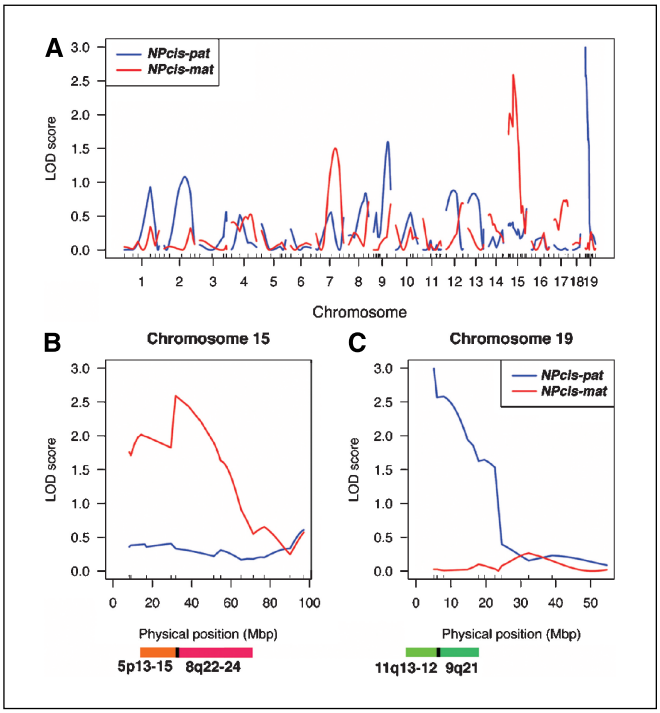

Figure 3. Linkage analysis of resistance to GEM PNST in (C57BL/6J × A/J) × C57BL/6J-*NPcis* and C57BL/6J × (A/J × C57BL/6J)-*NPcis* backcross progeny. *A,* LOD plot across all autosomes for backcross progeny. *Blue,* progeny of (C57BL/6J × A/J)-*NPcis* fathers and wt C57BL/6J mothers. *Red,* progeny of (C57BL/6J × A/J)-*NPcis* mothers and wt C57BL/6J fathers. *B,* linkage was found to chromosome 15 (LOD = 2.6, $P = 0.05$) for $NPcis^{mat}$ progeny. *Red bars under the peak,* syntenic regions of human chromosomes 15p13-15 and 8q22-24. *C,* linkage was found to chromosome 19 (LOD = 3.0, $P = 0.02$) for $NPcis^{pat}$ progeny. *Green bars under the peak,* syntenic regions of human chromosomes 11q12-13 and 9q21.

C57BL/6J × A/J-*NPcis* fathers to wt C57BL/6J mothers and compared the $NPcis^{pat}$ progeny to the $NPcis^{mat}$ progeny of C57BL/6J × A/J-*NPcis* mothers crossed to wt A/J fathers. In this experiment, the A/J strain background is inherited from the opposite parent as the *NPcis* mutant mouse chromosome 11, controlling for imprinted effects in the strain background as opposed to imprinted effects linked to mouse chromosome 11. In the reciprocal backcrosses, increased susceptibility to GEM PNSTs is again associated with inheriting *NPcis* mutant chromosome 11 from the father, not the A/J strain background ($P = 0.0002$, χ^2 test; Table 1). This evidence shows that the effect of the reciprocal crosses on tumor susceptibility is not due to the mitochondrial genome or to the maternal influences on the embryo because these effects would be dependent on strain differences. We conclude that an imprinted locus on mouse chromosome 11 affects susceptibility to GEM PNST.

Because the F1 crosses showed that polymorphic modifier loci can affect the susceptibility to GEM PNSTs (Fig. 2) and the reciprocal crosses showed that an imprinted locus on mouse chromosome 11 can also affect susceptibility to GEM PNSTs (Table 1), we wanted to determine whether there was any genetic interaction between the imprinted locus and the polymorphic loci responsible for the strain-specific effects. We identified the polymorphic modifier loci by binary trait linkage analysis of backcross progeny (14, 19). We chose the A/J strain as the resistant test strain because of the availability of the genome sequence for comparison to C57BL/6J (23), as well as the availability of chromosome substitution strains (24), to aid in future refinement of the modifier loci. We backcrossed C57BL/6J × A/J-*NPcis* to wt C57BL/6J mice and phenotyped the backcross progeny for the presence or absence of GEM PNSTs when the mice were sacrificed due to tumor burden. A total of 254 backcross progeny were phenotyped and genotyped at 105 simple sequence length polymorphism markers with an average sweep radius of 8 cM. Binary trait linkage analysis of the pooled backcross progeny found no significant linkage of simple sequence length polymorphism markers to resistance to GEM PNSTs. However, when the backcross progeny were split into $NPcis^{mat}$ and $NPcis^{pat}$ progeny, significant linkage was found on two different chromosomes (Fig. 3) using a permutation test to determine the genome-wide adjusted significance threshold (P value) of 5% or less.

In $NPcis^{pat}$ backcross progeny, resistance to GEM PNSTs maps to the simple sequence length polymorphism marker D19Mit59 at the proximal tip of mouse chromosome 19 ($P = 0.02$ by permutation test for genome-wide significance). We have named this locus *nerve sheath tumor resistance (Nstr) QTL 1* or *Nstr1*. Progeny that are homozygous for the C57BL/6J allele at *Nstr1* are more likely to develop GEM PNSTs, consistent with the presence of a resistance allele in the A/J strain (Table 2). Interestingly, this locus has no effect on the resistance of $NPcis^{mat}$ backcross progeny (Fig. 3; Table 2).

In $NPcis^{mat}$ backcross progeny, resistance to GEM PNSTs maps to simple sequence length polymorphism marker D15Mit111 on mouse chromosome 15 ($P = 0.05$ by permutation test for genome-wide significance; Fig. 3). $NPcis^{mat}$ progeny that carry the A/J allele at this locus are less likely to develop GEM PNSTs (Table 2), again consistent with the presence of a resistance allele in the A/J strain (Table 2). We have named this locus *nerve sheath tumor resistance (Nstr) QTL 2* or *Nstr2.* Whereas *Nstr1* on mouse chromosome 19 has little effect on $NPcis^{mat}$ progeny, *Nstr2* has little effect on $NPcis^{pat}$ progeny (Fig. 3; Table 2).

If a polymorphic modifier gene acts cell-autonomously and dominantly to block tumor formation, the allele responsible for the resistance is more likely to be lost when tumors form than the allele that does not confer resistance (25). To determine whether *Nstr1* and *Nstr2* are lost in tumors and are likely to act within the tumor cell during initiation, we characterized 20 of the rare GEM PNSTs from the resistant C57BL/6J × A/J-*NPcis*

Table 2. Affected *NPcis* backcross mice by genotype

Progeny	D15Mit111		D19Mit59	
	n	With GEM PNST* (%)	*n*	With GEM PNST* (%)
$NPcis^{pat}$, B6/A†	76	44 (58%)	58	25 (43%)
$NPcis^{pat}$, B6/B6‡	66	44 (67%)	72	54 (87%)
$NPcis^{mat}$, B6/A†	52	12 (23%)	52	21 (40%)
$NPcis^{mat}$, B6/B6‡	57	31 (54%)	48	18 (38%)

*With GEM PNST for a given genotype.
†B6/A indicates mice heterozygous for C57BL/6J and A/J at the specified marker.
‡B6/B6 indicates mice homozygous for C57BL/6J at the specified marker.

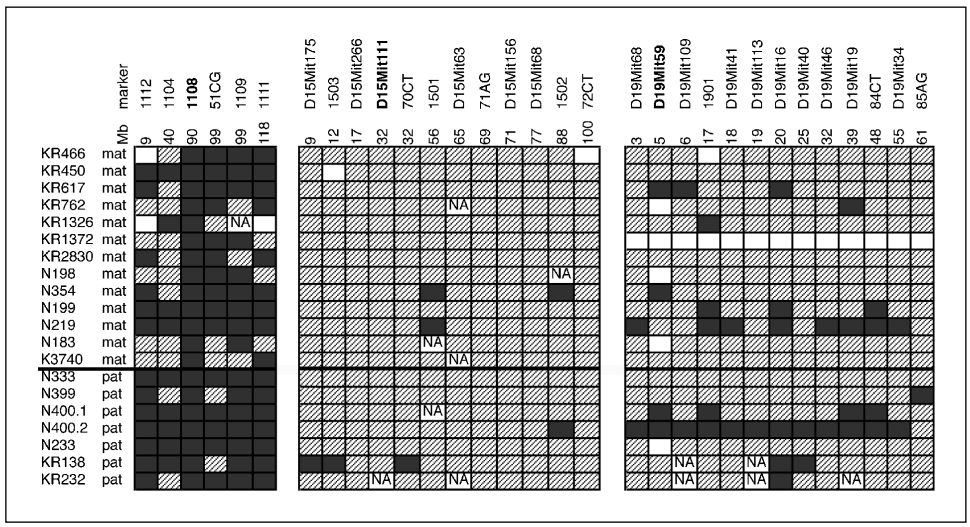

Figure 4. Allelic imbalance in C57BL/6J × A/J-*NPcis* F1 GEM PNSTs. Each row represents the genotype of a single tumor along the chromosome. *Black,* reduction in the A/J allele by >30%. *White,* reduction in the C57BL/6J allele by >30%. *Hatched,* samples scored as heterozygous. *NA,* samples that gave no data (not available). *Boldface,* markers nearest to the mapped loci; marker nearest to *Nf1* are indicated on chromosome 11.

background for allelic imbalance on mouse chromosome 15 and 19 (Fig. 4). MPNSTs are known to involve hyperproliferation of many stromal cell types, in addition to the tumor cells (1). We used quantitative methods to assess allelic imbalance because we expect the tumor samples to be contaminated with significant numbers of nontumor cells. Quantitative real-time PCR was used to analyze allelic imbalance at 110 single-nucleotide polymorphisms throughout the genome[9] and densitometry of ethidium stained gels was used to further analyze 15 simple sequence length polymorphism markers on mouse chromosomes 15 and 19 to confirm the results (Supplementary Fig. S1). We found no allelic imbalance on mouse chromosome 15 in the region of *Nstr2*. In the genome-wide single-nucleotide polymorphism scan for allelic imbalance, mouse chromosome 15 was the least affected of any chromosome, with 6 of 13 tumors analyzed showing small regions of allelic imbalance (Fig. 4). None of the tumors showed loss at simple sequence length polymorphism marker D15Mit111. Allelic imbalance was found on mouse chromosome 19, with 9 of 20 tumors examined showing imbalance. Of the nine cases of allelic imbalance at the simple sequence length polymorphism marker D19Mit59, four showed reduction of the A/J allele, consistent with loss of a resistance modifier allele from the A/J strain. On mouse chromosome 19, we observed allelic imbalance in tumors from both *NPcis*^{pat} mice (3 of 7 tumors examined) and *NPcis*^{mat} mice (6 of 13 informative tumors examined). Although one might expect *Nstr1* to be altered only in *NPcis*^{pat} tumors because *NPcis*^{mat} backcross progeny do not show linkage to *Nstr1*, the allelic imbalance measures the end state of the tumor whereas the linkage to *Nstr1* in the backcross progeny measures the rate-limiting step in tumorigenesis. Loss of *Nstr1* occurs in GEM PNSTs from both *NPcis*^{pat} and *NPcis*^{mat} but may only be rate limiting to tumorigenesis in *NPcis*^{pat} progeny. These data on allelic imbalance suggest that *Nstr1* shows allelic imbalance in roughly half of tumors examined with equal preference for loss of the A/J allele and the C57BL/6J allele.

We have used a haplotype prediction approach (26) to confirm that *Nstr1* and *Nstr2* carry polymorphic regions, consistent with our observations of different strain phenotypes, and to focus on regions of likely modifiers within the mapped loci. We have compared these regions to syntenic regions of the human genome to see if they are implicated in human MPNST tumorigenesis. Using available haplotype maps,[7] we have looked for regions where the resistant A/J strain has a different haplotype from the susceptible C57BL/6J and 129 strains (Supplementary Fig. S2). On mouse chromosome 15 between D15Mit201 and D15Mit151, three regions fit this pattern at 37.67, 41.92, and 44.57 Mb. At 41.92 Mb, A/J shares a haplotype with DBA/2J, another resistant strain, and at 44.57 Mb, A/J shares a haplotype with CAST/EiJ, which is similarly resistant. In addition, these regions are syntenic with human chromosome 8q22-23. Human chromosome 8q has been found to be amplified in MPNSTs (27, 28); a translocation between chromosome 5q11 and chromosome 8q22 (29) and a translocation between chromosome 5q13 and chromosome 8q23 (30) have been reported in MPNST; and amplification of chromosome 8q22 has been observed in an MPNST (31). Whereas these alterations are not common events in human MPNSTs, as might be expected for alterations in a highly penetrant tumor suppressor gene or oncogene, they are consistent with the presence of a low-penetrance polymorphic modifier gene. These data suggest that the syntenic regions of mouse chromosome 15 and human chromosome 8q22-23 contain a polymorphic modifier gene that affects GEM PNST and MPNST tumorigenesis.

A similar analysis of haplotype structure on mouse chromosome 19 between the centromere and D19Mit109 found one candidate region at 5.22 Mb, very close to the peak at D19Mit59 (Supplementary Fig. S2). At this location, the A/J strain shares a haplotype with DBA/2J. This region also overlaps with *Mtes1*, a modifier of breast cancer metastasis identified in mice (32). Interestingly, this region is syntenic with human chromosome 11q13, a region found to be specifically deleted in two MPNSTs (30, 33) and specifically amplified in one MPNST (33). In addition, translocations between human chromosome 11q13 and chromosome 15p11 (30), chromosome 19p13 (34), and chromosome 19q13 (30) have been reported. Taken together, these data are consistent with the presence of a polymorphic modifier gene affecting both GEM PNSTs and MPNSTs on proximal mouse chromosome 19 and human chromosome 11q13.

[9] S. Tsang, Z. Sun, J. Diehl, K. Reilly, and D. Munroe, in preparation.

Discussion

The data we present here, together with our previous data (10), show that *NPcis* mice are more susceptible to GEM PNSTs or more susceptible to astrocytoma depending on whether the *NPcis* mutation is inherited from the father or the mother, respectively. We confirm here that these effects are independent of strain background and are therefore linked to inheritance of mouse chromosome 11 rather than to strain-specific factors such as maternal cytoplasm or mitochondria. The effect of mouse chromosome 11 on susceptibility is likely due to changes in dosage of monoallelically expressed genes during tumor initiation. An imprinted gene expressed from the wt mouse chromosome 11 may be lost when the wt copies of *Trp53* and *Nf1* are lost during tumor initiation. An imprinted gene expressed from the *NPcis* mutant chromosome 11 may be reduplicated through subsequent genome instability after loss of *Trp53*. Alternatively, the chromatin marks that maintain the silenced chromosome at the imprinted locus may change the genomic stability on mouse chromosome 11, such that the imprinted chromosome is more or less likely to be lost or mutated. Recent data in *NPcis* mice have shown increased loss of wt mouse chromosome 11 when the genome is hypomethylated through mutations in *Dnmt1* (35). This raises the possibility that the differential methylation on the two parental chromosomes at imprinted loci may affect their rates of loss. Whether the imprinted locus drives the loss of wt *Trp53* and *Nf1* or whether the mutation at *Trp53* and *Nf1* drives the loss or duplication of the imprinted locus remains to be determined. However, this distinction has important implications for targeting therapies against imprinted genes, depending on whether the effect of the imprinted locus is through effects on chromatin structure or through effects on gene dosage.

The reciprocal effect of mouse chromosome 11 inheritance on susceptibility to astrocytoma and GEM PNST is interesting and raises questions about the nature of the imprinted gene causing the effect. A recent study has identified 37 candidate imprinted genes on mouse chromosome 11 using a bioinformatics approach (36), in addition to the two confirmed imprinted genes on mouse chromosome 11, *Grb10* (37, 38) and *U2af1-rs1* (39). It is possible that there are two unrelated imprinted genes on mouse chromosome 11 affecting astrocytoma and GEM PNSTs independently, with opposite imprinting. Alternatively, a single imprinted gene on mouse chromosome 11 may act as an oncogene in one tissue type and as a tumor suppressor gene in the other, such that, for example, loss of the paternal chromosome leads to increased tumorigenesis in one tissue and reduplication of the paternal chromosome leads to increased tumorigenesis in the other. Another possibility is that a single gene is expressed from different parental chromosomes in the two tissue types but has a similar function (as an oncogene or tumor suppressor gene) in the two tissues. In the example of an imprinted tumor suppressor gene, loss of the wt paternal mouse chromosome 11 would lead to increased astrocytoma whereas loss of the wt maternal mouse chromosome 11 would lead to increased GEM PNST. It is interesting to note that *Grb10* has been shown to be expressed from the paternal chromosome in the brain and from the maternal chromosome in other organs in a highly isoform-specific manner (37, 38). Finally, differences in gene silencing on mouse chromosome 11 between the two tissues types may cause sequestering of the maternal or paternal chromosome. This may lead to differences in the mutation rate or kinetics of chromosome loss for a particular parental chromosome, leading to a secondary effect on the rate of mutation of the wt copies of *Nf1* and *Trp53*.

The importance of epigenetic changes in human cancer is now well established (see ref. 12 for review) with changes in dosage levels of imprinted genes such as *IGF2* (40, 41) affecting the rate of tumorigenesis. The monoallelic expression of imprinted genes acts similarly to the first "hit" in Knudson's two-hit model of cancer (42), with the second hit occurring either through epigenetic mechanisms (loss of imprinting) or through genetic mechanisms. An example of genetic changes acting to alter imprinted gene expression was first shown in human rhabdomyosarcomas (43) where maternal human chromosome 11p15 is preferentially lost and paternal human chromosome 11p15 is duplicated. More recently, susceptibility to succinate dehydrogenase D (*SDHD*)–linked paraganglioma was found to be inherited by paternal transmission of the mutant *SDHD* gene. Because *SDHD* is biallelically expressed, it has been proposed that a linked imprinted gene on human maternal chromosome 11 is lost when the wt allele of *SDHD* is lost. In this way, inheritance of *SDHD* mutation from the father has a long-distance effect on a linked imprinted gene. Our data in the *NPcis* mouse model show a very similar effect, with concomitant loss of the wt copy of *Trp53* and *Nf1* with another imprinted locus on mouse chromosome 11.

We have analyzed here for the first time the genetic interaction between an imprinted locus and polymorphic loci and its effects on cancer susceptibility. The imprinted locus on mouse chromosome 11 interacts epistatically with a network of other loci to affect resistance to GEM PNSTs. In the case of *NPcis*^{pat} mice with increased susceptibility to GEM PNSTs, a locus on chromosome 19 on the A/J strain background acts to increase resistance. In the case of *NPcis*^{mat} mice with reduced susceptibility, a locus on chromosome 15 on the A/J strain background further increases the resistance to GEM PNST. The relevance of these results to human NF1 patients is supported by the overlap of these loci with regions altered in MPNSTs. Consistent with these loci being low-penetrance modifiers for MPNST susceptibility, we see evidence for changes in these genomic regions in a relatively low number of mouse and human tumors. However, the observation that these regions are translocated in multiple human tumors suggests that these rearrangements are not random. Identification of modifiers of NF1 in mouse models will allow these modifiers to be tested directly in human association studies, specifically in cases where tumors can be tested for the change in expression of candidate imprinted genes. These data show that polymorphic modifier genes affect tumorigenesis under very specific conditions. The understanding of these conditions will allow for more accurate risk assessment in the future and genetic counseling for individuals at high risk for cancer.

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