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Source: Radiation Research, 157(4):426-434.
Published By: Radiation Research Society
DOI: http://dx.doi.org/10.1667/0033-7587(2002)157[0426:BTIBAP]2.0.CO;2
URL: http://www.bioone.org/doi/full/10.1667/0033-7587%282002%29157%5B0426%3ABTIBAP%5D2.0.CO%3B2

RADIATION RESEARCH **157**, 426–434 (2002)
0033-7587/02 $5.00

Bone Tumorigenesis Induced by Alpha-Particle Radiation: Mapping of Genetic Loci Influencing Predisposition in Mice

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Rosemann, M., Lintrop, M., Favor, J. and Atkinson, M. J. Bone Tumorigenesis Induced by Alpha-Particle Radiation: Mapping of Genetic Loci Influencing Predisposition in Mice. ***Radiat. Res.*** **157, 426–434 (2002).**

The present study was carried out to determine the extent to which genetic factors modify the incidence of radiation-induced bone tumorigenesis in mice, and to map putative susceptibility genes. We conducted a genome-wide linkage analysis in a cohort of 47 interstrain backcrossed mice. After the mice were injected with the bone-seeking α-particle-emitting radionuclide ^{227}Th, 21 of the mice developed osteosarcomas. Two loci, one on chromosome 7 close to D7Mit145 and a second on chromosome 14 (D14Mit125), exhibited suggestive linkage to osteosarcoma predisposition, with LOD scores of 1.37 and 1.05, respectively. The LOD score increased considerably when interaction between these two loci was taken into account (LOD = 3.48). Nine of 12 mice inheriting a susceptibility allele at both loci developed osteosarcomas after ^{227}Th injection, compared to only four osteosarcomas in 18 animals that did not inherit either of the susceptibility alleles. Variance component analysis revealed that these genetic factors determine approximately one-fifth of the total incidence of osteosarcomas. This study demonstrates the presence of a genetic component that modulates predisposition to radiation-induced osteosarcoma.

INTRODUCTION

Osteosarcoma is a relatively rare form of cancer, accounting for less than 2% of all primary malignancies in humans (*1*). However, bone tumors in general, and osteosarcomas in particular, were among the earliest neoplasms to be causally linked to radiation exposure. The first clear evidence for this was found among U.S. radium dial painters exposed to long-lived ^{226}Ra and ^{228}Ra isotopes (*2, 3*). Another radium isotope (^{224}Ra) used for therapeutic purposes because of its short half-life of only 3.64 days was also associated with an increased incidence of osteosarcomas in a long-term follow-up study in ankylosing spondylitis patients (*4, 5*). In a more recent epidemiological study, an increased incidence of bone tumors was reported among Russian plutonium workers (*6*), again confirming that osteotropic α-particle emitters in general confer a high risk of bone tumors. Many of the biological and pathological mechanisms underlying the high efficiency of bone-seeking α-particle emitters in inducing osteosarcoma have been elucidated in experimental animals such as dogs (*7*) and mice (*8*). These studies have established the influences of dose, fractionation and age at exposure. Since radiation-induced osteosarcomas appear to be very similar in mice and in humans in terms of skeletal localization and histology (*9*), it is also likely that similar molecular alterations are involved in their pathogenesis.

Although osteotropic α-particle emitters have the clearest effect on bone tumor incidence, epidemiological studies also show an increase in the incidence of osteosarcoma after external irradiation. Patients treated in childhood with radiotherapy or combined radiotherapy/chemotherapy for leukemia carry an increased risk for osteosarcoma later in life (*10–13*). Although osteosarcomas are over-represented among radiotherapy-associated secondary neoplasias, it cannot be ruled out that this is because of a general propensity for the development of tumors in patients who have had childhood leukemia (*14*). A clear distinction between a pure radiation etiology and a genetic predisposition in these patients cannot be made due to the case–control approach of the study (*15*). Thus it would be of interest to distinguish between these two factors in a model system in which the incidence of radiation-induced osteosarcoma can be compared in cohorts of randomly selected genotypes.

The relevance of an inherited predisposition for spontaneous or radiogenic osteosarcoma is difficult to estimate. It is already known that germline mutations resulting in an inactive form of the *RB1* (*16, 17*) or *TP53* (*18*) tumor suppressor genes confer an increased risk for both spontaneous and radiogenic osteosarcoma. From the observed numbers of osteosarcoma patients carrying these two mutations (*16, 19*), one can estimate that not more than 8% of all the cases diagnosed are associated with germline mutations of these genes. The existence of additional genetic factors which predispose to osteosarcoma is indicated by the occurrence

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of familial or multifocal bone tumors in patients with no evidence of germline *RB1* or *TP53* mutations (*20–23*). Candidates for such additional genetic factors include putative osteosarcoma suppressor genes mapping to human chromosomes 3q and 18q (*24, 25*). There are also reports of bone cancer developing in siblings without any indications of a cancer-prone syndrome in the parental generations (*21, 26, 27*). Furthermore, differences in the incidence of osteosarcoma in different ethnic groups have been reported (*28, 29*); these may be indicative of what is commonly called a ''genetic background effect''.

Recently it was postulated that cosegregation of multiple low-penetrance modifier genes might have a significant influence on tumor predisposition (*30*). Due to the complex inheritance pattern, the genes involved in such a trait would be less easy to detect, but might be responsible for a large proportion of all ''sporadic'' tumors.

Exhaustive genetic linkage studies to map susceptibility genes for radiation-induced osteosarcoma in humans are not feasible because of the small number of cases and the lack of large affected sibling cohorts. As an alternative strategy, we have mapped genetic factors responsible for the differences in susceptibility to radiogenic osteosarcoma in different inbred strains of mice (*31*). We have exploited the potent effect of the bone-seeking α-particle emitter ^{227}Th in inducing osteosarcoma (*8, 32*) and have conducted a whole-genome linkage analysis with microsatellite markers to map susceptibility loci.

MATERIALS AND METHODS

Treatment and Maintenance of Mice

The animal experiment was approved by the responsible governmental authorities of the State of Bavaria (file number 211-2531-56/90). The study was funded by the European Union (grant no. F14P-CT95-0001), and the data were accepted by EULEP and DOE for their joint International Radiobiology Archives of Long-Term Animal Studies. All mice were cared for in compliance with the national animal protection law. The progress of the study was reported regularly to the local veterinary authorities in Munich, who also inspected the animal facilities several times a year.

C3H/HeJ and 102/E1 mice were obtained from GSF breeding stocks. The T-stock was developed by W. L. Russell (*33*) by outcrossing inbred NB mice to a non-inbred stock and extracting by further breeding mice homozygous recessive at seven marker loci. The stock has been maintained by random mating to avoid inbreeding. A population of 47 female mice, obtained from T-stock $\times$ (C3H/HeJ $\times$ 102/El) F_1-hybrid breeding, was treated i.p. with 35 kBq/kg ^{227}Th (as thorium citrate) at the age of 100 days. Incorporation of the isotope was measured 20 days after injection using a Canberra Packard whole-body counter mounted to a germanium crystal detector. A group of 69 mice from the same breeding stock were sham-irradiated and served as control animals. All mice were housed about five to a cage and were examined 5 days a week. Moribund animals, or those with palpable tumors, were killed humanely and subjected to a detailed necroscopy. Bone tumors were diagnosed radiologically and were confirmed by histological examination after EDTA decalcification.

DNA Extraction and Microsatellite Genotyping

DNA was extracted from liver tissue by incubating frozen and pulverized tissue in 1% SDS and 2 mg/ml proteinase K for 24 h at 37°C and was purified using a semi-automatic DNA processor (Autogen 540, Biometra, Germany). In four cases (two mice with and two mice without osteosarcoma), DNA was extracted from 10 μm histological sections (muscle, heart, liver or spleen) using the QuiaAmp DNA extraction kit (Quiagen GmbH, Hilden, Germany). PCR amplification was carried out according to the protocol recommended for MIT microsatellite marker (*34, 35*) using a TC1 Thermal Cycler (Perkin Elmer Cetus, Boston, MA). PCR products were size-fractionated by gel electrophoresis in 3% agarose/TBE with ethidium bromide staining. Informative markers were defined as those showing a visible size difference between the C3H and the 102 alleles, with at least one of them being distinct from the maternal T-stock alleles.

Statistical Analysis

Analysis of body weight and thorium incorporation was performed using Student's *t* test. Genetic linkage analysis was carried out by scoring the inheritance of either the C3H or 102 allele for each microsatellite marker. Differences between the two groups of mice (with or without osteosarcoma) in the inheritance patterns at single markers were then determined using the χ^2 test. LOD scores for a single-locus trait model were calculated using the LINKAGE software package (*36*) and for a two-trait-locus model using the TMLINK program (*37, 38*). In contrast to other programs which calculate linkage between single marker genotypes and a particular phenotype, TMLINK yields likelihood estimates for linkage of the compound genotype at two mutually unlinked markers with one phenotype. For this purpose, two disease loci are assumed, which are being tested simultaneously for linkage to one of the two markers. The two-marker-locus LOD score is then calculated as

$$\log_{10}\frac{L(\theta_1, \theta_2)}{L(0.5, 0.5)},$$

where θ_1 and θ_2 are the recombination frequencies of the two pairs of marker and disease loci and L is the likelihood of linkage assuming binomial distribution of the alleles and recombination frequency as specified. Penetrances and age-dependent liability classes must be derived *a priori* from a segregation study; they are listed in Table 3.

Differences in lifetime osteosarcoma incidences between mice with different genotypes were tested for significance using the log-rank test (*39*). The genetic component of the variation in osteosarcoma incidence was assessed following the general procedure of Falconer (*40*) as follows: Phenotypic variation (V_P) was computed from the tumor incidence p of the total cohort as the binomial variance $p \times (1 - p)$, treating the entire cohort as a mixed population. Environmental variation (V_E) was computed as the weighted average of the corresponding binomial variances of the four possible genotypic sub-cohorts, considering each sub-cohort to be a uniform population, and with the weights being the relative size of each cohort. The contribution of genetic variation, V_G, to susceptibility to osteosarcoma was deduced by subtracting the environmental variation component from the entire phenotypic variation, i.e. $V_G = V_P - V_E$.

RESULTS

Osteosarcoma Incidence is not Influenced by Thorium Retention or Body Weight

Of 47 mice injected with ^{227}Th at the age of 100 days, 21 developed bone tumors, with a mean latent period of 652 ($\pm$116) days (Fig. 1). Radiological and histological examinations show that all tumors included in this study were osteoblastic osteosarcomas (Fig. 2). In the remaining 26 animals treated with ^{227}Th that had a similar life span after

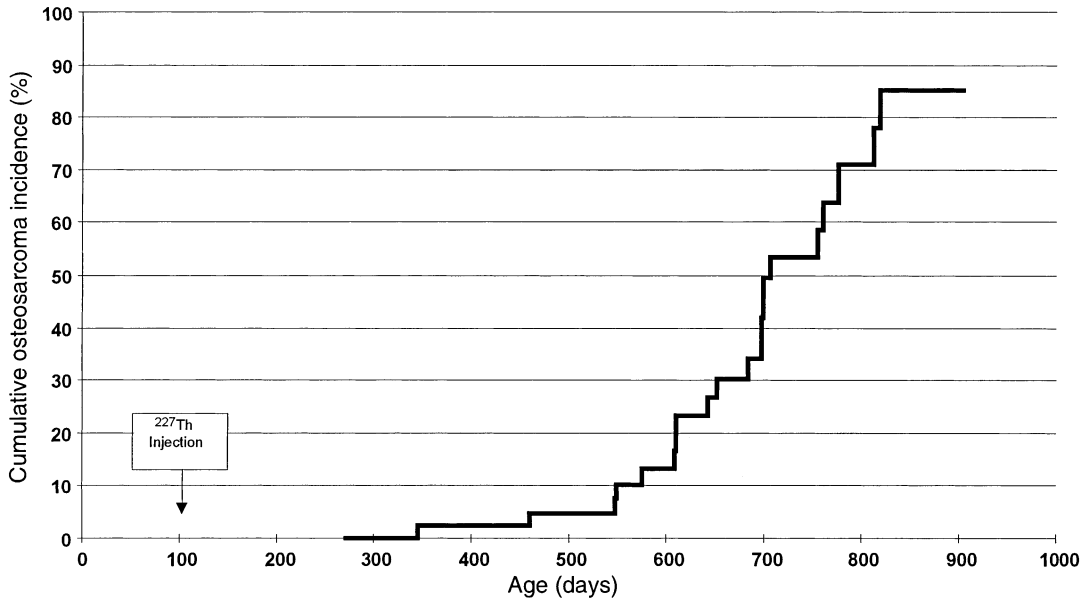

FIG. 1. Cumulative osteosarcoma incidence (corrected for competing causes of death) after injection of 47 mice from a T-stock × (C3H × 102) F_1 cross with 35 kBq/kg ^{227}Th at the age of 100 days.

injection (593 ± 161 days), no osteosarcomas were evident at the time of death. Neither body weight at the time of injection (27.5 ± 2.5 g in osteosarcoma-bearing mice, 29.5 ± 3.5 g in osteosarcoma-free mice, difference not significant), nor ^{227}Th retention (average retention 0.71 ± 0.06 kBq/kg and 0.68 ± 0.04 kBq/kg, respectively, difference not significant) could account for the observed segregation of susceptibility to osteosarcoma. One of the 69 sham-irradiated mice developed a bone tumor, but due to its exceptionally long latent time of 964 days, it was not included in the subsequent linkage study.

Two Regions on Chromosome 7 and 14 Determine Osteosarcoma Susceptibility

The 102/El inbred mouse strain used in these studies was originally derived by a recombinant inbred cross between C3H/HeJ and 101/Rl strain animals (www.informatics.jax.org/external/festing/mouse/STRAINS.shtml). Screening with a set of 177 microsatellite markers that distinguishes between C3H and 101 alleles revealed that approximately 43% of the 102 strain genome is actually derived from the C3H strain (Fig. 3). Consequently, segregation of C3H and 102 alleles in the T-stock × (C3H × 102) cross can occur

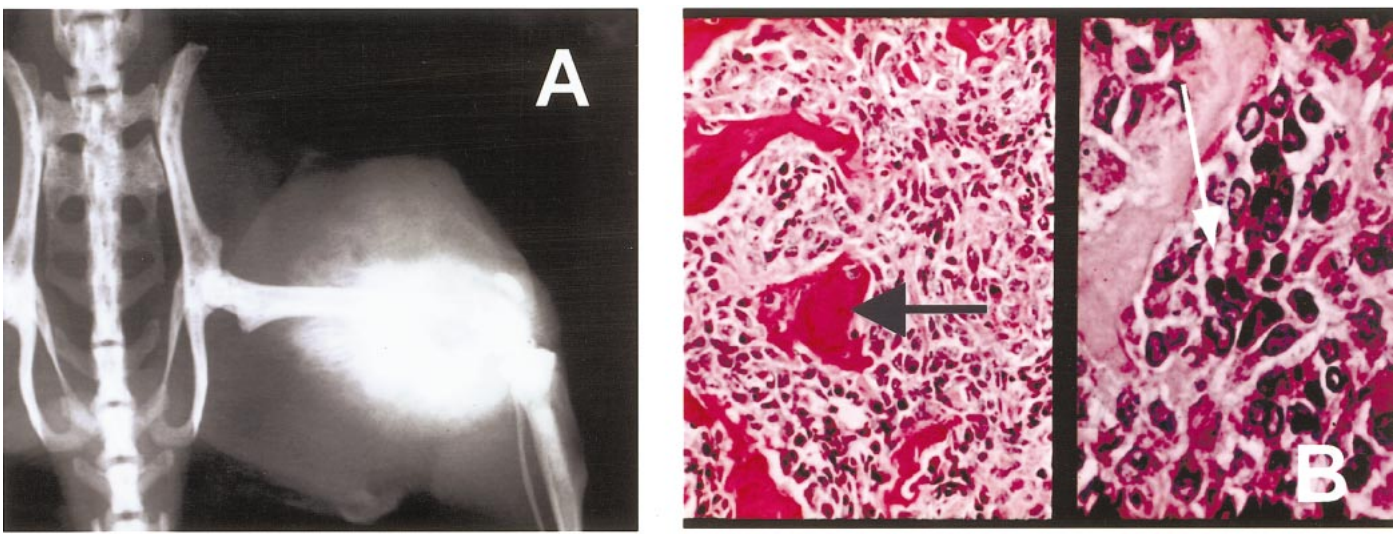

FIG. 2. Panel A: Radiological image of a typical ^{227}Th-induced osteosarcoma developing from the distal end of the femur. Note the X-ray dense tumor tissue, reflecting the high degree of mineralization. Panel B: Histological section of the same tumor after decalcification and staining with hematoxylin and eosin. Note the presence of osteoid (large arrow) and atypical osteoblast cells (small arrow), evidence for osteoblastic osteosarcoma.

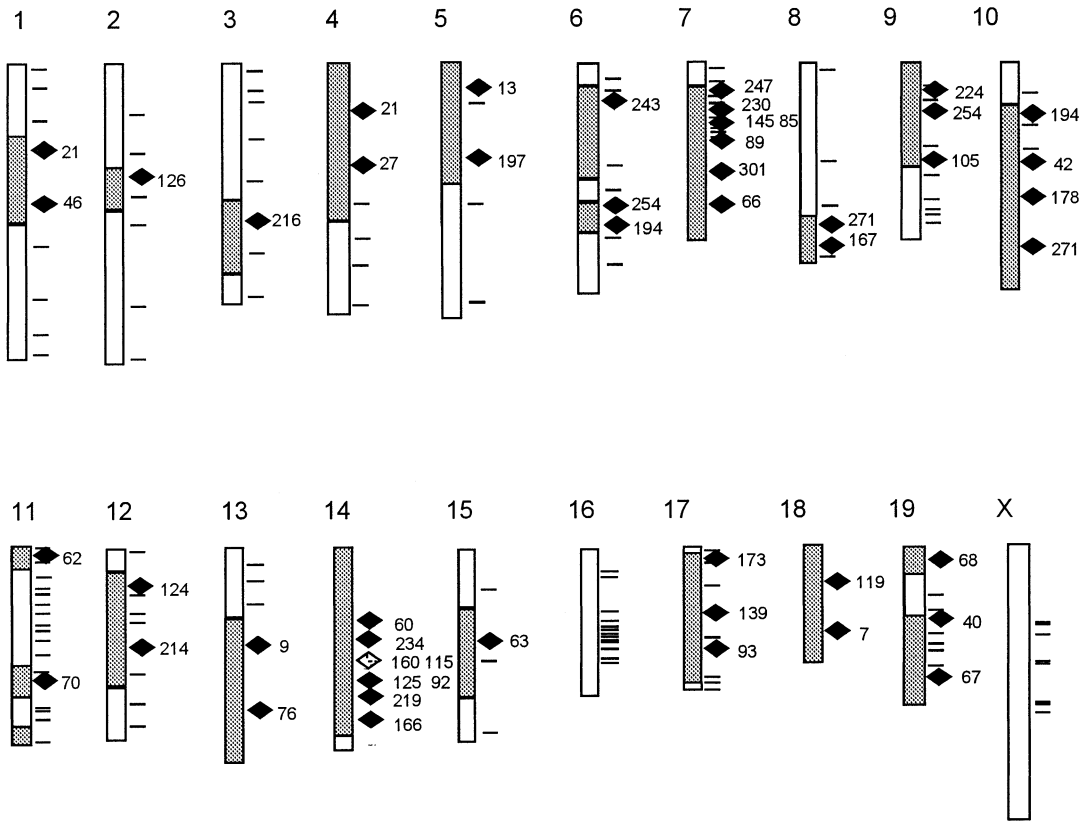

FIG. 3. Ideogram of the genome of the 102 mouse strain showing regions originally derived from the C3H strain (open bars) and from the 101 strain (filled bars). Positions of microsatellite markers used for linkage analysis are shown as black diamonds. Microsatellite marker positions used for allelotyping the C3H- and 101-derived regions are depicted as small lines.

only in the remaining 57% of the genome, for which all mice were subsequently genotyped using a set of 54 microsatellite markers. From all markers analyzed, only those mapping to two genomic regions on chromosomes 7 and 14 exhibited a statistically significant difference in allele segregation between the tumor-bearing and tumor-free mice. Markers D7Mit85, D7Mit145 and D7Mit89 on chromosome 7 were linked with osteosarcoma predisposition, with the 102 allele occurring in 18 of the 26 tumor-free mice but in only 7 of the 21 mice that developed a tumor ($P = 0.014$, LOD = 1.37, Table 1). This LOD score is considered to be indicative of genetic linkage (*41*), and it suggests the presence of a tumor resistance allele in the 102 strain. On chromosome 14, the strongest linkage was observed for markers D14Mit125 and D14Mit219 ($P = 0.036$ and 0.033, respectively; LOD = 1.05), with the 102 allele present in 13 of the 21 mice with osteosarcoma but in only 8 of the 26 tumor-free mice (Table 1). The LOD score for this locus is also indicative of linkage, but here the 102 allele is associated with tumor susceptibility.

Inheritance of Susceptibility Alleles at both Regions Confers a Threefold Increase in Osteosarcoma Incidence

Seventy-five percent of the mice inheriting a susceptibility allele at both loci (haplotype D7Mit145^{C3H}, D14Mit125^{102}) developed osteosarcoma. In contrast, mice carrying a resistance allele at both loci (haplotype D7Mit145^{102}, D14Mit125^{C3H}) had an osteosarcoma incidence of only 22% ($P = 0.001$, log rank test; Table 2 and Fig. 4). Mice with a resistance allele at one locus and a susceptibility allele at the second locus were of intermediate susceptibility, with incidences of 50% (D7Mit145^{C3H}, D14Mit125^{C3H}) and 43% (D7Mit145^{102}, D14Mit125^{102}) for the two genotypes.

Estimating the effect of each locus separately revealed that inheritance of the 102 allele on chromosome 7 conferred reductions in tumor incidence of 28% in D14Mit125^{C3H} mice and 32% in D14Mit125^{102} mice. Conversely, inheritance of the 102 allele at the locus on chromosome 14 resulted in an increase in the tumor incidence of 21% in D7Mit145^{102} mice and 25% in D7Mit145^{C3H} mice (Table 2).

Interaction between both Susceptibility Loci

On a linear scale, the absolute effect of the 102 allele on either locus appears to be independent of the allelic state at the other locus. The increase in incidence caused by the 102 allele on chromosome 14 is 21% in D7^{102} mice and 25% in D7^{C3H} mice, and reduction of incidence by the 102 allele on chromosome 7 is 28% in D14^{C3H} mice and 32% in D14^{102} mice, respectively). To test for a possible complex inheritance pattern of the phenotype that does not show up

TABLE 1
Microsatellite Marker on Two Chromosomal Regions Showing Positive Linkage with Osteosarcoma Predisposition

Marker	Map position (cM)	No. 102 heterozygotes/no. mice: Mice without tumors	Mice with osteosarcomas	*P* value (χ^2 test)	LOD
D7Mit230	24.6	16/26	7/21	0.054	0.8
D7Mit85	25	18/26	7/21	0.014	1.37
D7Mit145	25	18/26	7/21	0.014	1.37
D7Mit89	27	18/26	7/21	0.014	1.37
D7Mit301	46.4	15/26	9/21	0.310	0.23
D7Mit66	57.5	12/26	7/21	0.370	0.12
D14Mit160	46.5	7/26	9/21	0.252	0.38
D14Mit115	47.5	7/26	9/21	0.252	0.38
D14Mit92	52.9	7/26	11/21	0.074	0.78
D14Mit125	52.5	7/26	12/21	0.036	1.05
D14Mit219	53.6	8/26	13/21	0.033	1.05
D14Mit166	62.3	8/26	12/21	0.070	0.78

Notes. Genotyping to determine inheritance of the 102 or C3H allele was carried out in 21 mice that developed osteosarcoma after ^{227}Th injection and in 26 mice that remained osteosarcoma-free. *P* values were calculated using the χ^2 test. LOD scores were calculated using the LINKAGE program. Map positions of the markers were taken from the MIT database (*20*).

on a linear scale, a two-locus trait model (*37, 38*) was used. The age-dependent liability classes and associated osteosarcoma penetrances used for this calculation were derived from the incidence curves (Fig. 4) and are listed in Table 3. The compound Chr7/Chr14 genotypes exhibit linkage to osteosarcoma, with a LOD score of 3.48, equivalent to a *P* value of 6×10^{-5} (*41*). This is considerably higher than the arithmetic sum of the two single-locus LOD scores (2.42) and indicates epistatic interaction between two putative osteosarcoma susceptibility genes.

Approximately One-fifth of the Osteosarcoma Variation is due to a Genetic Component

Partitioning the osteosarcoma variance components as described in the Materials and Methods yields a value for the entire phenotypic variance (V_P) of 0.25 and for the environmental component (V_E) of 0.20. The residual variance ($V_P - V_E$) is therefore 0.05, or one-fifth of the entire phenotypic variance, and reflects the genetic component influencing the trait (*40*) (Table 2).

DISCUSSION

Bone tumorigenesis after the incorporation of bone-seeking α-particle emitters or high-dose external irradiation is an established late effect in both humans and experimental animals. In patients treated with radiotherapy early in life, osteosarcomas are one of the most frequent secondary neoplasms observed. However, it is not clear to what extent an underlying genetic disposition could influence the observed frequency of apparent radiation-induced osteosarcoma (*15*).

Here we analyzed strain-dependent differences in inbred mice with different levels of susceptibility to osteosarcoma after α-particle irradiation. We investigated whether, and to what extent, defined genetic loci influence predisposition. A genome-wide linkage analysis was carried out in a T ×

TABLE 2
The Four Different Cohorts of T-stock × (C3H × 102) F_1 Mice Represented by Their Genotype at D7Mit145 and D14Mit125 Together with the Combined Population, with Their Cohort Sizes, Number of Diagnosed Osteosarcomas, and (Uncorrected) Cumulative Incidence

		Haplotype			
	Total	$D7^{C3H}$ $D14^{102}$	$D7^{C3H}$ $D14^{C3H}$	$D7^{102}$ $D14^{102}$	$D7^{102}$ $D14^{C3H}$
Number of mice	47	12	10	7	18
Mice with osteosarcoma	21	9	5	3	4
Osteosarcoma incidence	45%	75%	50%	43%	22%
Variance	0.25			0.2	

Note. V_P, the phenotypic variance of the combined cohort, is the result of genetic plus environmental/epigenetic variability, computed as the usual binomial variance for the total cohort.

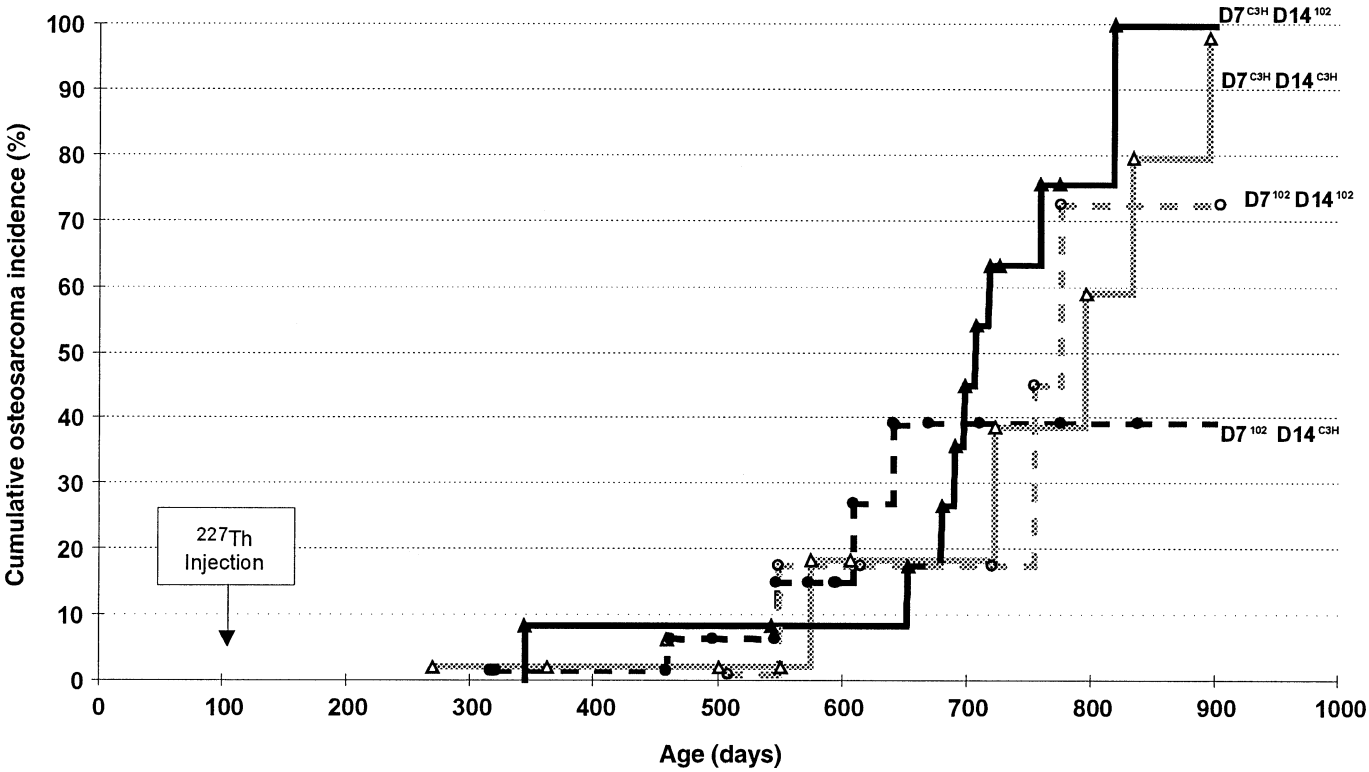

FIG. 4. Cumulative osteosarcoma incidence (corrected for competing causes of death) after injection of mice with 35 kBq/kg ^{227}Th at the age of 100 days. Genotypes at D7Mit145 and D14Mit125 were determined for 47 mice from a T-stock × (C3H × 102) F_1 cross, yielding four distinct groups of animals, each characterized by the compound genotype at the two loci. The difference between the incidence curves of D7Mit145^{102}D14Mit125^{C3H} and D7Mit145^{C3H}D14Mit125^{102} is statistically significant ($P = 0.001$, log-rank test). Pairwise differences between other incidence curves are not significant (i.e. $P > 0.05$).

(C3H × 102) interstrain cross. Using the osteotropic α-particle-emitting radionuclide ^{227}Th to induce bone tumors, we identified two loci, on chromosomes 7 and 14, which harbor putative susceptibility genes for osteosarcoma. Mice inheriting susceptibility alleles at both loci developed osteosarcoma with an incidence of 75% compared to an incidence of only 22% in mice carrying the resistance alleles at both loci. It is worth noting that a 102 allele confers susceptibility at the locus on chromosome 14 but that a 102 allele is associated with resistance at the chromosome 7

TABLE 3
Age-Dependent Liability Classes and Calculated Osteosarcoma Penetrances as Used for the TMLINK Program

		Liability class:	1	2	3	4
		Age (days):	1–400	401–550	551–700	701–900
Genotype						
D7^{102}D14^{C3H}	No. of mice		2	5	7	4
	No osteosarcomas		0	1	2	1
	Penetrance		0	0.20	0.29	0.25
D7^{C3H}D14^{C3H}	No. of mice		2	2	5	1
	No osteosarcomas		0	0	4	1
	Penetrance		0	0	0.8	1
D7^{102}D14^{102}	No. of mice		0	2	1	4
	No osteosarcomas		0	1	0	2
	Penetrance		0	0.5	0	0.5
D7^{C3H}D14^{102}	No. of mice		1	2	5	4
	No osteosarcomas		1	1	5	2
	Penetrance		1.00	0.50	1.00	0.50
All genotypes	No. of mice		5	11	17	13
	No osteosarcomas		1	3	11	6
	Penetrance		0.20	0.27	0.65	0.46

Note. Values are derived from the incidence curves of the four two-locus genotypes as shown in Fig. 4.

locus. Thus mice with mixed C3H or 102 genotypes at both loci exhibit more pronounced phenotypes in terms of osteosarcoma susceptibility than mice with the parental C3H or 102 genotype at both loci.

LOD scores calculated for each of the two loci separately were 1.37 and 1.05. Adopting the statistical criteria outlined by Lander and Kruglyak (*41*), these LOD scores must be considered to be only suggestive of linkage. However, the stringent criteria for reporting linkage as proposed above were challenged by others, since in complex genetic traits, interaction of multiple genes could partially mask linkage to the single loci (*42, 43*). Gene interaction during tumorigenesis is well known for certain molecular pathways: Aberrant cyclin D expression and mutations/deletions in *RB1* and *CDKN2A* (also known as *p16*) appear to be mutually exclusive in certain tumors, reflecting the interaction of these genes within the pathway regulating the G_1/S-phase transition (*44–46*). In contrast, a coincidence between losses of functional *TP53* and *RB1* is often found in osteosarcoma and other tumor types, showing that two pathways must be defective for tumor initiation (*47, 48*). Although these well-proven examples apply to somatic changes during tumorigenesis, it is also likely that epistatic interactions are involved in congenital cancer predisposition (*49*). To test for this, we applied a genetic two-locus-trait model to estimate linkage between the compound genotype at D7Mit145/D14Mit125 and osteosarcoma susceptibility. Such a sequential approach, i.e. the identification of ''promising'' loci by a single-marker linkage screen followed by the analysis of two-locus interaction of all pairs of markers with an initial positive LOD score, was proposed by Ott (*50*) for complex genetic patterns in human pedigrees. With this approach for D7Mit145/D14Mit125 compound genotypes, linkage increased significantly to LOD = 3.48, demonstrating that the observed trait of osteosarcoma predisposition is strongly influenced by an interaction of putative susceptibility genes in the regions of the marker loci.

According to Lander and Kruglyak (*41*), this LOD score corresponds to a *P* value of 6×10^{-5}. If we adopt a threshold for whole-genome significance of $\alpha_{WG} = 0.05$, a threshold of $\alpha_{ST} = 9.5 \times 10^{-4}$ must be used for each single test (Bonferroni correction). It thus can be concluded that linkage of the compound D7Mit145/D14Mit125 genotype with osteosarcoma predisposition is highly suggestive.

Partitioning the osteosarcoma variance into a genetic and a non-genetic (i.e. environmental) component revealed that genetic predisposition is involved in approximately one-fifth of all osteosarcoma cases. This is considerably higher than the estimates for the genetic component for spontaneous osteosarcoma in humans, for which germline mutations in two genes, *RB1* and *TP53*, account for no more than 8% of all cases. This could indicate the presence of unknown types of alterations in these tumor suppressor genes that modify tumor predisposition. Alternatively, the larger level of genetic influence found in our study could indicate that genetic factors have a stronger impact on susceptibility to radiation-induced bone tumors than on spontaneous bone tumors.

Susceptibility genes are assumed by definition to be only partially penetrant. This, together with the small number of mice analyzed in the present study, precludes the direct identification of the susceptibility genes. Nevertheless, it is possible to nominate or exclude candidate genes using the available human-to-mouse synteny maps (www.ncbi.nlm.nih.gov/Homology/). Four tumor suppressor gene loci implicated in the development of osteosarcomas in humans have been mapped to chromosome 3q (*24*) chromosome 13q (*RB1*) (*51*), chromosome 17p (*TP53*) (*52*), and chromosome 18q (*25*). The *TP53* gene, germline mutations of which are associated with an increased risk of developing spontaneous osteosarcoma (*18*), maps to mouse chromosome 11 in a region that does not segregate in this study. The *RB1* gene, which has been implicated in the etiology of both spontaneous and radiation-induced osteosarcoma in humans (*16, 17*), maps 11 cM proximal to D14Mit125 and hence lies within the chromosome 14 region we have identified as conferring susceptibility to osteosarcoma. Since neither mouse strain used in these studies demonstrates abnormalities associated with a homozygous loss of *Rb1* function (*53, 54*), one would expect only minor differences between the C3H and 102 allele of the *Rb1* gene.

The two remaining suppressor loci in humans, on chromosomes 3q and 18q, map to syntenic regions of mouse chromosomes 9 and 18, respectively. The fact that neither locus shows linkage to the development of radiation-induced osteosarcoma in the mouse strains examined here, however, is not completely unexpected. One could argue that each gene or locus involved in spontaneous bone tumor development does not necessarily have to be involved in the susceptibility to the same type of tumor after radiation exposure. Alternatively, it is also possible that the segregating C3H and 102 alleles at the these loci does not confer a phenotypic difference and that linkage is thus obscured. Crosses between inbred mice can detect linkage only for a certain subset of all loci, depending on the genealogical relationship of the strains under study (*55*).

Candidate genes close to the linked region at 25 cM on chromosome 7 would include *Bax* (23 cM from centromere), *Xrcc1* (5.5 cM from centromere), and *Fosb* (5 cM from centromere). Given the epistatic interaction between the loci on chromosomes 7 and 14, an unidentified gene on chromosome 7 could act as a modifier gene for the osteosarcoma-predisposing effect of *Rb1* on chromosome 14.

We demonstrate here for the first time that genetic factors can influence disposition to the development of radiation-induced osteosarcoma in a mouse model. Given the similarities in the etiology, histology and pathology of osteosarcoma in mice and humans, it is not unreasonable to predict that the susceptibility loci identified using the mouse model will also play a role in the development of osteosarcoma in humans.

ACKNOWLEDGMENTS

The authors greatly appreciate the detailed histological evaluations of all tumors by Prof. A. Luz. We also thank Elke Hartmann, Eleonore Samson, J. Muller and Anna Nickel for their technical assistance and J. Ott (Rockefeller University, Laboratory of Statistical Genetics) for fruitful discussions concerning the two-locus trait model. Parts of this project were funded by grant F14P-CT95-0001 from the radiation protection program of the European Union. M. Lintrop received a scholarship award from the Swedish Radiation Protection Institute.

Received: March 6, 2001; accepted: November 16, 2001

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