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Multilocus inheritance determines predisposition to α-radiation induced bone tumourigenesis in mice

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In a recent study, we presented evidence for genetic predisposition governing radiation osteosarcomagenesis in mice. Following the incorporation of the bone-seeking α emitter ^{227}Th, ~25% of the variance in osteosarcoma incidence was determined by inherited genetic factors. We have now mapped 5 susceptibility loci in crosses between the more susceptible BALB/c and the more resistant CBA/Ca strains. The major QTL on chromosome 14 overlaps with a locus that was already found in our previous study, using different strains of mice. Here, we investigate the effect by which the major susceptibility locus and 4 minor modifier loci interact to influence osteosarcoma predisposition. Following incorporation of the bone-seeking isotope, 100% of mice that harbour high-risk genotypes at all 5 susceptibility loci develop osteosarcoma with an average of 472 days latency times. In 10 mice inheriting exclusively low-risk genotypes only 1 osteosarcoma was found, arising after 733 days latency time. Inheritance of distinct combinations of BALB/c and CBA/Ca alleles at the susceptibility loci confer more extreme phenotypes in terms of susceptibility or resistance than observed in either of the two parental inbred strains. From the present study, we demonstrate that additive effects of multiple alleles, each making only a minor phenotypic contribution, can combine and significantly alter tumour risk. This mechanism can be of particular importance in genetically heterogeneous populations such as man.
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Key words: osteosarcoma; radiation; carcinogenesis; genetic susceptibility

Osteosarcoma (OS) is the most important late effect following incorporation of osteotropic α emitters such as Radium or Thorium.[1] They were among the first tumours with a clearly demonstrated radiogenic aetiology in man, following occupational or therapeutic incorporation of radium isotopes[2–4]. OS is also over-proportionally frequent among secondary tumours, arising after radiotherapy or combined radio-chemotherapy.[5–7] Congenital predisposition to spontaneous or radiogenic OS is encountered in some rare inherited conditions such as Li-Fraumeni, familial Retinoblastoma, Werner's syndrome and Rothmund-Thompson syndrome, where predisposition can be increased several 100-fold. Fortunately, the mutant alleles that are responsible for these highly-penetrant congenital disorders have a low population frequency, and one can thus estimate that the portion of all diagnosed bone tumours related to any of these conditions is below 10%. Association studies of tumours in close relatives suggest, however, that a considerable fraction of assumed sporadic OS might in fact be linked to multiple low-penetrance genetic variations.[8] Such multigene interaction could lead to compound genotypes that confer a significantly increased tumour risk, both spontaneously as well as following radiation exposure.[9] Mapping and identification of modifier genes that govern congenital cancer susceptibility in inbred mouse strains have been demonstrated following tumour induction by chemical carcinogens or targeted germline mutations[10–13]. For tumours following radiation exposure, mapping of susceptibility loci has been performed for lymphoma and leukaemia.[14–17]

In a previous study, we found evidence for 2 discrete loci on mouse chromosome 7 and 14, which in combination confer an increase in α-particle induced OS incidence from 22 to 75%.[18] These 2 loci account for about one-fifth of the entire tumour susceptibility, with some indications for nonadditive genetic interaction. The present study was intended to identify additional or confirm previously mapped loci by using other strains of inbred mice. In particular, we have focused on the degree by which genetic factors can alter predisposition for radiogenic OS.

Material and methods

Animal breeding and tumour induction

All animal procedures were carried out in accordance with federal animal welfare guidelines. Inbred BALB/c and CBA/Ca mice were obtained from GSF breeding stocks. Backcrossed mice (B1) were produced by mating female BALB/c to male (BALB/c × CBA/Ca) F1 hybrid mice. At the age of 100 days, 84 female BALB/c mice, 63 female CBA/Ca mice, 80 female BALB/c × CBA/Ca F1 hybrid mice and 169 female B1 mice were injected with a single i.p. dose of 185 Bq/g ^{227}Th (as Thorium Citrate). This activity accumulates to give a mean α-dose of 6 Gy to the entire skeleton. Details of the ^{227}Th synthesis, its dosimetry and bio-physiological properties are described elsewhere.[19] Twenty days after injection, the incorporated thorium activity was measured for each mouse using a Gamma Vision 32 counter (Ortec, Oak Ridge, TC) mounted on a NaI scintillation detector 19SW12/W3 (Harshaw, Cleveland, OH). For control purposes, 230 female mice from the same breeding colonies (40 BALB/c, 40 CBA/Ca, 50 F1 and 100 F2) were left untreated. Animals were housed 5 to a cage and examined 5 days a week for the development of tumours. Bone tumours were diagnosed radiologically, and confirmed by histological examination after EDTA decalcification. Typical histologies and X-ray images of bone tumours have been deposited at the Pathbase® database (URL: http://www.pathbase.net, Acc. no. PB3410, PB3411, PB3412, PB3414).

DNA extraction and microsatellite genotyping

Genomic DNA was extracted from 5-mm tail tips, as previously described.[18] Microsatellite genotyping was done by PCR using 20 ng genomic DNA, 5 pmol forward and reverse primer, rTAQ-poly-

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Abbreviations: B1, first backcross; F1, hybrid cross; LRS, likelihood-ratio statistics; LT_C, normalized censored latency time; OR, odds ratio; OS, Osteosarcoma; QTL, quantitative trait locus; 5xS, compound 5 locus susceptibility; 5xR, compound 5 locus resistance.

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merase, reaction buffer with 1.5 mM $MgCl_2$ (Amersham Pharmacia, Freiburg, Germany). Amplification in 96-well plates using a GeneAmp 9700 thermocycler (Applied Biosystems) was followed by 90 min electrophoresis on 3% agarose/TBE gels. Primer sequences and reaction conditions were used as recommended by others.[20]

Linkage analysis

For a whole genome linkage analysis, 53 polymorphic DNA markers for BALB/c and CBA/Ca were selected such that the distance from any locus in the mouse genome to the nearest marker was less than 18 cm (Fig. 2). This ensures that a putative susceptibility locus conferring at least a 23% modification of tumour incidence will be detected with 85% power (Log-Rank Test). For this initial linkage screen, a selective genotyping approach was used by comparing tumour-free mice that reached a minimum age with mice that developed OS up to a maximum latency time (Table II). Two-point linkage analysis was performed for each single marker, using χ^2 test. For chromosomal regions showing significant deviations from a random distribution of heterozygote BALB/CBA and homozygote BALB/BALB genotypes among tumour-bearing and tumour-free mice, 21 additional informative markers were selected to further define the locus. QTL interval mapping was done using MapManager QTXb20. This program calculates a likelihood-ratio statistic (LRS = 4.6 × LOD) for a marker interval to harbour a QTL.[21] Threshold LRS that qualify for suggestive or significant linkage with a whole-genome significance of 0.05 were computed using the permutation test. As trait variable for QTL analysis, each mouse was assigned a normalized and censored tumour susceptibility LT_C calculated as follows:

$$LT_C = (t_{max} - t)/t_{max} \text{ (mice diagnosed with OS)}$$
$$LT_C = -t/t_{max} \text{ (mice dying without OS)}$$

with t being the age at death and t_{max} being the longest observed survival time (813 days in our study).

LT_C thus runs from –1 to 0 for OS-free mice and from 0 to 1 for mice that developed OS. LT_C values of –1 represent mice that remained tumour-free for a maximum life span, thus exhibiting highest resistance. Values close to 1 are assigned to mice with tumours arising early in life, thus representing susceptible animals. Values around 0 are assigned to mice that either died young without OS or developed tumours with long latency times, both cases representing animals with less reliable phenotype. This parameter can also be considered as a dichotomous trait (tumour *vs*. tumour-free) multiplied with a weighting-factor that reflects lower or higher reliability of the phenotype.

Tumour incidence curves corrected for confounding causes of death were calculated using the Miescher algorithm. Incidence curves were truncated when the number of survivors declined below 10%, thus avoiding a bias from small group sizes. Log-rank significance tests for differences in OS kinetics were done using Statistika 6.0 (StatSoft, Tulsa, OK).

Results

Strain differences in OS incidence

The development of OS following ^{227}Th incorporation is significantly different in the BALB/c and CBA/Ca inbred strains. Following injection of 185 Bq/g ^{227}Th, equivalent with about 6 Gy skeletal dose, 28 of 84 BALB/c mice (33.3%) develop OS as compared to the CBA/Ca strain, where only 9 of 63 mice (14.3%) were diagnosed with this tumour type. The difference between the latency times was less pronounced, with a median latency of 520 days in BALB/c-mice and of 549 days in CBA/Ca mice. The difference between the corrected OS incidence curves of BALB/c and CBA/Ca (Fig. 1*a*) is statistically highly significant ($p = 0.0044$, log-rank test). Our study confirms an earlier observation of a strain difference.[22] F1 hybrid mice exhibited an intermediate tumour incidence with 23 of 80 animals (28.75%) being diagnosed with OS and 572 days median latency time. OS incidence curves in the two inbred strains BALB/c and CBA/Ca and the F1 cohort seem to follow a similar kinetics but shifted to longer or shorter latency times (suppl. mat. S1).

Of the 169 backcrossed BALB/c × (BALB/c × CBA/Ca) mice, 33 (19.5%) developed OS. Main tumour sites were ilium/sacrum, femur/tibia, vertebral column and humerus (suppl. mat. S2). Smallest tumours (1.5–5 mm) were diagnosed in the vertebral column, and largest tumours (8–20 mm) were found in the os ilium and os sacrum. Multiple tumour sites were diagnosed in 11 mice. All OS

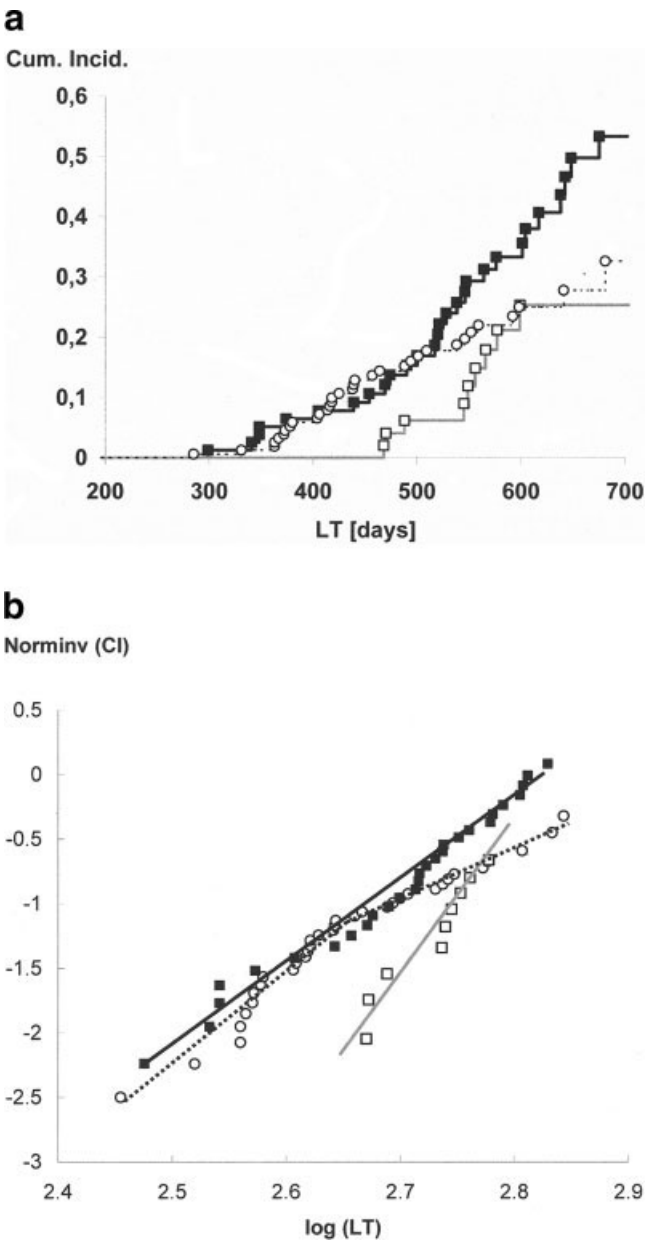

Figure 1 – (*a*) OS incidence curves in BALB/c (——■——), CBA/Ca (——□——) and backcrossed BALB × (BALB × CBA) mice (B1) (---○---) following incorporation of 185 Bq/g ^{227}Th at the age of 100 days. Plots are corrected for confounding causes of death, using the Miescher algorithm. (*b*) Log-normal plot of OS incidences in BALB/c (——■——), CBA/Ca (——□——) and B1 mice (···○···). Quantiles of corrected OS incidences (assuming normal distribution) are plotted against logarithm of the latency times. Regression curves are least-square estimates over the entire time range (BALB/c and CBA/Ca) or broken down into two intervals (B1) ranging from 0 to 460 days and from 461 to 700 days.

TABLE I – INCORPORATED 227 Th ACTIVITIES AND BODY WEIGHTS (EACH AS MEAN VALUE AND 95% CONFIDENCE INTERVAL) AS MEASURED 20 DAYS AFTER THORIUM-INJECTION IN DIFFERENT COHORTS OF MICE

	Spec. activity mean (Bq/g)	CI	Body weight mean (g)	CI
BALB/c	3.54	2.44–4.63	21.89	17.91–25.86
CBA/Ca	4.08	3.29–4.88	20.73	17.15–24.32
F1	4.06	3.25–4.86	22.24	17.86–26.61
B1	4.01	2.96–5.06	22.92	18.84–27.01
B1 OS-free	4.03	2.99–5.06	22.95	18.80–27.09
B1 OS	3.96	2.85–5.06	22.82	19.02–26.61

F1: BALB/c × CBA/Ca hybrid mice.
B1: BALB/c × F1 backcross mice.
Data for B1 mice are further subdivided into those of tumor-free mice and those of mice that later developed osteosarcoma.

used for our study were of an osteoblastic type, with variable degrees of osseous differentiation. The later was classified as "highly differentiated" with tumours showing osteoid synthesis in more than 80% of their area, "moderately differentiated" for tumours showing osteoid synthesis in 30–80% of the area and "poorly differentiated" for tumours showing only immature osteoid synthesis in an otherwise cell-rich tumuor-mass (suppl. mat. S3).

The tumour incidence with time follows a different kinetics in the backcrossed B1 cohort as compared to the genetically homogenous inbred and hybrid cohorts (Fig. 1*a*). In the log-normal plot of the incidence curves, the B1 backcrossed mice goes in parallel with the sensitive BALB/c strain up to 520 days after ^{227}Th injection. At later time points, however, the curve approaches that of the relatively resistant CBA/Ca strain (Fig. 1*b*). This peculiarity is also obvious in the distribution of tumour latency times, which are similar in CBA, BALB/c and F1 mice, but show a remarkable distortion toward shorter latency times in the backcrossed B1 cohort (suppl. mat. S4). The variable kinetics and latency times of bone tumourigenesis in the heterogeneous B1 population suggest that segregation of multiple independent genes from both parental strains govern OS development. In 2 out of 245 untreated mice bone, tumours were found at necroscopy, which is in accordance to earlier observations of a lower sporadic OS rate in NMRI mice.[23]

Lack of association between tumourigenesis and variability in thorium retention or weight

Table I gives mean values and 95% confidence intervals of body weight and incorporated ^{227}Th activity in the two inbred strains BALB/c and CBA/Ca, in the F1 hybrid mice and in the B1 backcrossed cohort. For the B1 cohort, the values were calculated separately for mice that later developed an OS and for those that remained tumour-free. No significant differences were detected between the various cohorts or between mice of different tumour status for either of the two parameters. This indicates that genetic variation in bone-tumour susceptibility is not expressed as a variation in the ^{227}Th retention of the isotope or as variation in body weight.

Genetic linkage analysis of OS susceptibility

The chromosomal positions of the microsatellite-markers (Fig. 2) used for genotyping the 169 B1 mice were obtained from the Mouse Genome Database (http://www.informatics.jax.org/searches/marker_form.shtml). This shows that, except for three regions on chromosome 2 and 3, each about 12 cm and for a minor region on chromosome 11 (about 5 cm), the entire genome was covered by the markers (coverage 97.1%, based on an observed genetic length of the entire genome of 1360 cm[20]). Because of the outcross–backcross protocol (see material and method), the X chromosome does not segregate in our study.

With the initial linkage screen, 5 loci were detected, at which segregation of the BALB/c and CBA/C alleles in tumour-free and tumour-bearing mice exhibited a clear deviation from randomness (Table II). BALB/c homozygosity associates with predisposition for OS at D4Mit172 (chromosome 4, 8.6 cm, $p = 0.019$, $\chi^2 = 6.11$, OR = 5.6), D5Mit24 (chromosome 5, 60 cm, $p = 0.014$, $\chi^2 = 5.99$, OR = 3.8) and D12Mit157 (chromosome 12, 37 cm, $p = 0.006$, $\chi^2 = 8.77$, OR = 5.4). BALB/CBA heterozygosity predisposes for OS at D14Mit125 (chromosome 14, 44.3 cm, $p = 0.00009$, $\chi^2 = 15.23$, OR = 9.88) and at D18Mit119 (chromosome 18, 16 cm, $p = 0.008$, $\chi^2 = 6.3$, OR = 2.9).

QTL-mapping with additional markers at these loci confirmed that D14Mit125 is significantly linked to OS susceptibility (peak LRS = 15.4, equivalent to LOD = 3.34, Fig. 3). The threshold value for significant linkage is LRS > 11.4 and for suggestive linkage LRS > 5.4, as calculated using the MapManager permutation test. Although loci at chromosome 4, 5, 12 and 18 would thus only qualify for suggestive linkage (Fig. 3), they will later be used to estimate their phenotypic effect in combination with the major QTL at chromosome 14. Genetic linkage to OS separated according to their skeletal site was generally weaker than for all tumour sites combined, most probably due to the smaller number of cases in each group. The only exception from this was the locus on chromosome 5 between D5Mit1 and D5Mit34. Here, the peak LRS for OS restricted to the vertebrae reaches 14.1 (equivalent to LOD = 3.06) as compared to just 9.3 for all tumour sites combined. Additional tumour specific parameters such as size, differentiation state, or tumour multiplicity were not linked to any specific genotype.

The major susceptibility locus on chromosome 14 (D14Mit125) partially overlaps with the radiation-induced OS susceptibility locus identified in an earlier study.[18] The χ^2 of 15.23 for D14Mit125 in the present study is equivalent to a LOD score of 3.31, which together with data from the afore mentioned study results in a compound value of LOD = 4.4. According to generally accepted criteria for linkage studies,[24] such a value can be considered highly significant.

Phenotypic effect of single and multiple susceptibility alleles

Of the 5 loci that are linked with overall OS susceptibility, the strongest effect was found for D14Mit125. Mice inheriting the BALB/CBA genotype are characterized by an earlier onset of OS appearance (incidence curve of D14Mit125$^{BALB/CBA}$ mice approaches 20% at 457 days compared to 641 days in D14Mit125$^{BALB/BALB}$ mice) and by a higher cumulative incidence (57.4% in D14Mit125$^{BALB/CBA}$ *vs.* 31.7% in D14Mit125$^{BALB/BALB}$, Fig. 4). Homozygosity for BALB/c alleles was found associated with an increased OS risk at 3 of the 5 susceptibility loci. At the remaining 2 loci, however, heterozygosity for the CBA allele was seen to predispose to OS. This implies that offspring with distinct combinations of maternal and paternal genotypes would be more susceptible (*e.g.*, 5xS for D4Mit172$^{B/B}$ D5Mit24$^{B/B}$ D12Mit157$^{B/B}$ D14Mit125$^{B/C}$ D18Mit119$^{B/C}$) or more resistant (*e.g.*, 5xR D4Mit172$^{B/C}$ D5Mit24$^{B/C}$ D12Mit157$^{B/C}$ D14Mit125$^{B/B}$ D18Mit119$^{B/B}$) than either of the parental inbred strains. Although mice with these complex genotypes represent only about 6.7% of the entire backcross population, a remarkable difference in their OS susceptibility is evident (Fig. 5). As compared to the incidence curves of the two parental strains BALB/c and CBA/Ca, the 5 × S genotypes exhibit 100% incidence and the shortest tumour latency time. Among 10 offspring inheriting the 5 × R genotype, only one developed a tumour with an exceptionally long latency time of 733 days.

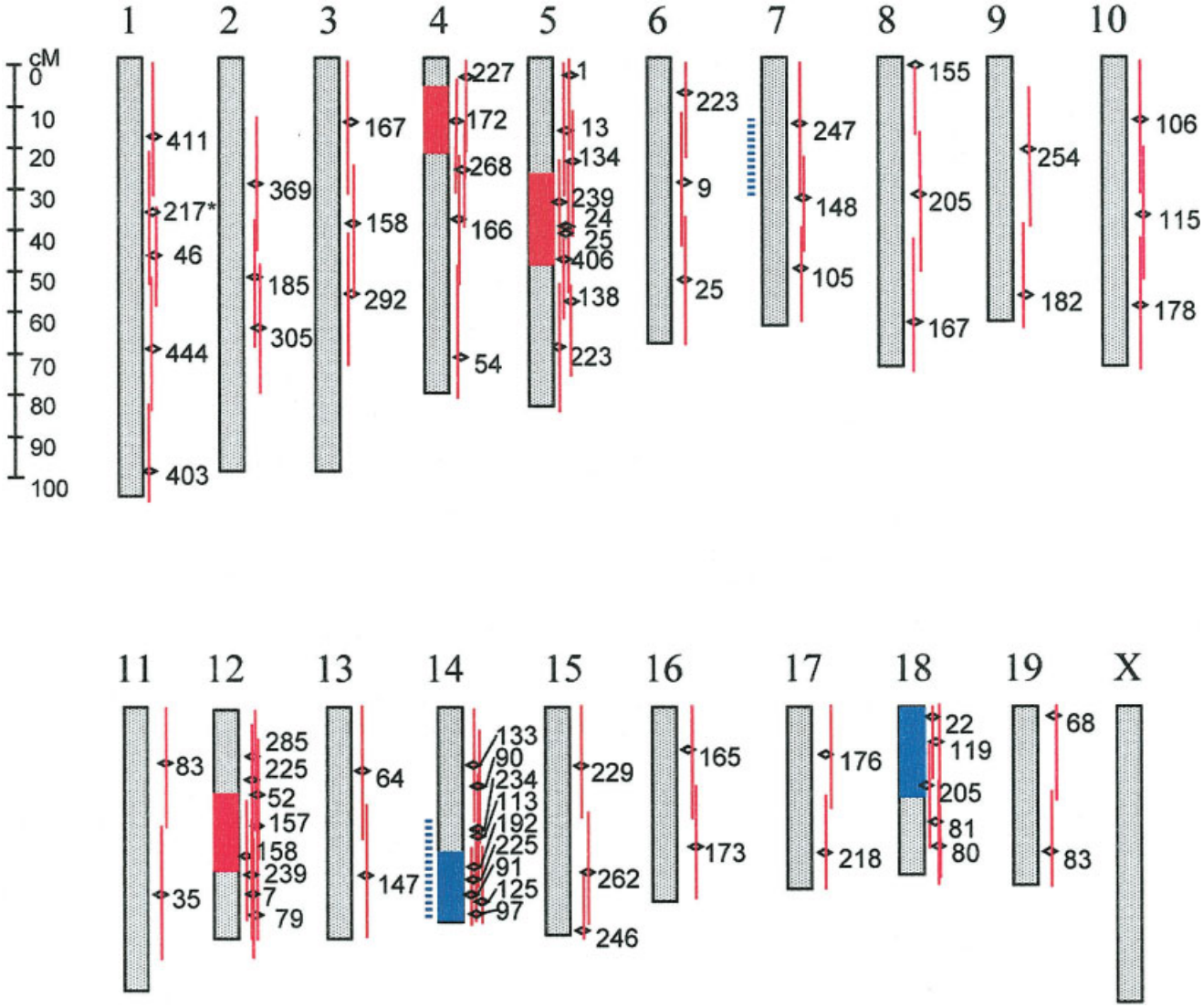

Figure 2 – Genomic position of marker loci, and approximate positions (derived from MGD database) of BALB/c/CBA polymorphic microsatellite markers are given as black diamonds. Numbers are related to the name of each marker in the MIT panel (* exception is 217 that was originally D3Mit217, but has now been allocated to chromosome 1). Red lines indicate the sweep-radius of each marker, that is, the interval for which linkage to susceptibility genes could be detected. Chromosomal regions showing linkage of OS susceptibility with BALB/c alleles (red) and CBA/Ca alleles (blue) are shown together with two OS susceptibility loci mapped in preceding study (hatched blue bars).

TABLE II – LINKAGE OF OSTEOSARCOMA SUSCEPTIBILITY TO SINGLE MARKERS AS FOUND BY THE INITIAL WHOLE-GENOME SCREEN

Marker	χ^2	OR	95% CI	*p*	High-risk genotype	OS/LT<	Non-OS/LT>
D4Mit172	6.11	5.60	1.37–23.1	2.00E−02	BALB/BALB	450	650
D5Mit24	5.99	3.83	1.28–11.5	1.40E−02	BALB/BALB	500	600
Dl2Mit157	8.72	5.40	1.7–17.4	6.00E−03	BALB/BALB	500	600
Dl4Mit125	15.23	9.88	2.9–33.7	9.53E−05	BALB/CBA	500	600
Dl8Mit119	6.30	2.90	1.25–7.1	1.21E−02	BALB/CBA	600	500

Association of high- and low-risk genotypes with number of osteosarcoma developing with a maximum latency of OS/LT< and number of osteosarcoma-free mice reaching a minimal age of Non-OS/LT> was tested using Chi2-Test.

Odds-ratios are given as mean (OR).

Discussion

Thorium is a rare earth-metal that, following oral incorporation, specifically binds to the surface of mineralized bone matrix. The radioactive isotope ^{227}Th has a physical half-life of 16.2 days, and thus deposits almost all of its α-particle energy into a small volume at the bone surface. This area includes the bone forming osteoblasts, bone resorbing osteoclasts as well as mesenchymal stem-cells and committed osteoblast precursor cells. ^{227}Th has long been used to experimentally induce bone-tumours in dogs[25] and mice,[26] with the later representing a suitable model for radiation-induced bone-tumours in man.[1] In humans, an occupational

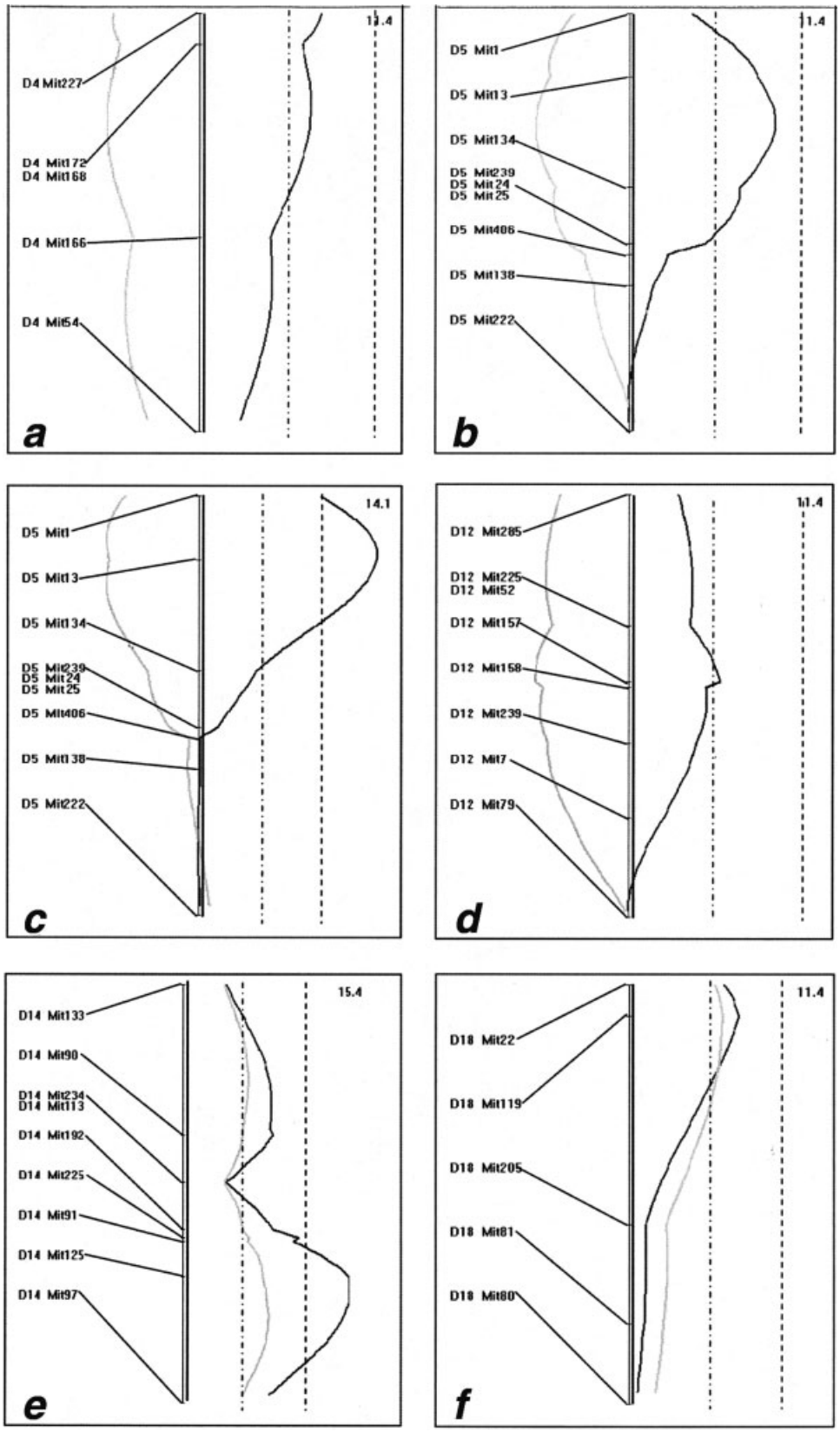

FIGURE 3 – QTL interval mapping. Mapping of OS susceptibility QTLs using the censored normalized tumor susceptibility LT_c as quantitative trait (see Materials and methods). Loci that exceed the threshold for suggestive linkage on chromosome 4 (*a*), chromosome 5 (*b*,*c*), chromosome 12 (*d*), chromosome 14 (*e*) and chromosome 18 (*f*) are shown. (*c*) shows QTL for susceptibility to OS of the vertebrae only. LRS (black curve) and additive effect (grey curve) were calculated using MapManager QTX. Thresholds for suggestive (dash-dot line) and significant linkage (dashed line) were calculated using MapManager permutation test. Number in the upper right corner of each panel gives the peak LRS value (*c*,*e*) or the value of the significance treshold (other panels).

or therapeutic ingestion of radium, thorium or other osteotropic α emitters can cause a tremendous increase in the frequency of bone tumours.[2–4,27] The first observation of their carcinogenic potential was found among U.S. radium dial-painters,[2,3] who ingested considerable amounts of the long lived Radium 226 and Radium 228. Among workers who had incorporated the most carcinogenic dose of the two isotopes, almost 50% developed OS. The spontaneous incidence of this tumour is exceptionally low

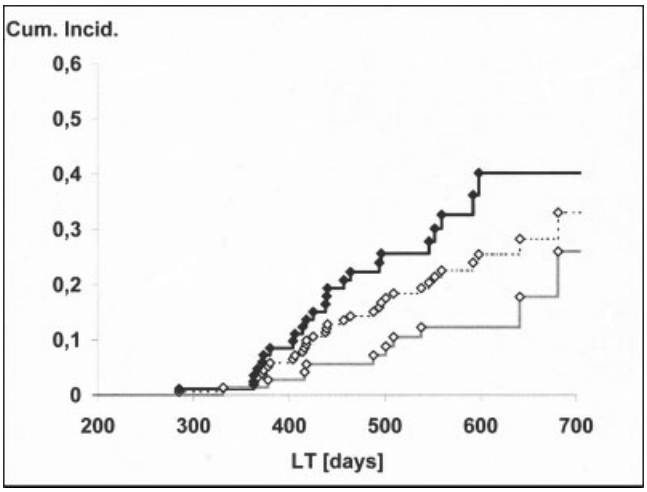

FIGURE 4 – OS incidence curves in D14Mit125 BALB/BALB and BALB/CBA mice. Corrected cumulative OS induction among 169 backcrossed B1 mice grouped according to their genotype at D14Mit125. $D14Mit125^{B/C}$ (——♦——), $D14Mit125^{B/B}$ (——◇——) and pooled genotypes (···◇···).

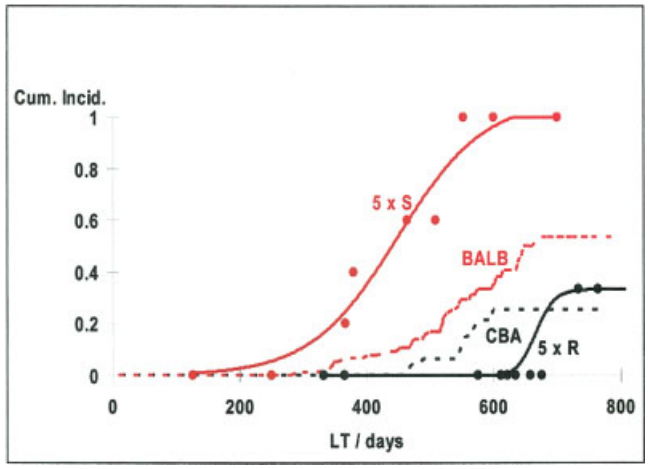

FIGURE 5 – OS incidence curves in mice of 5-locus compound genotypes. Corrected cumulative OS induction in backcrossed B1 mice with the compound genotypes (D4Mit172B/B D5Mit24B/B D12Mit157B/B D14Mit125B/C D18Mit119B/C = 5xS, red) and (D4Mit172B/C D5Mit24B/C D12Mit157B/C D14Mit125B/B D18Mit119B/B = 5xR, black) as compared to the incidence curves of the two inbred strains BALB/c (red dotted line) and CBA/Ca (black dotted line). Plots for the two inbred strains are redrawn from Figure 1*a*. Curves for the 5xS and 5xR cohorts are least square fits of a sigmoidal curve to the data points.

(only about 1 per 200,000 people per year) and no other exogenous factor apart from radiation is known to contribute to bone tumourigenesis.

Following ^{224}Ra or ^{227}Th injection into laboratory mice, differences in OS susceptibility among inbred strains were reported,[22,28,29] suggesting that genetic factors can influence predisposition. We have shown in an earlier study that linkage analysis in mouse crosses following injection with bone-seeking α emitters is a suitable tool for mapping genetic loci that harbour putative susceptibility genes. The small number of animals used in our study (derived from the inbred strains C3H/H Nhg and 102/Eh) yielded suggestive linkage for 2 loci on chromosome 7 and 14.[18] Here, we extended the linkage analysis by using two additional strains of mice, BALB/c and CBA/Ca, to determine if susceptibility loci are indeed common amongst the different genetic backgrounds of mice. In total, 5 susceptibility loci were found, exhibiting different degrees of linkage. The principal susceptibility locus at D14Mit125 on chromosome 14 (LOD = 3.31, OR = 9.88) colocalizes with 1 of 2 loci that were found in the previous study in C3H and 102 mice. The compound LOD score for this locus using data from the previous and the present study is now 4.4, and can thus be considered highly significant for linkage. The genotype at this locus influences both overall OS incidence and tumour latency (Fig. 4). OS incidence and latency time of the two groups of B1 mice, defined by the high- and low-risk genotype at D14Mit125 (Fig. 4), are well within the range of the OS incidences of the two parental inbred strains BALB/c and CBA/Ca (Fig. 1*a*). There is, however, a small subset of animals that, due to random segregation at the other 4 loci, inherit exclusively high-risk (5 × S) or low-risk alleles (5 × R). Animals with these compound genotypes show more extreme phenotypes than either of the parental inbred strains (Fig. 5).

Of the different tumour parameters that were tested for linkage, only the censored latency time exhibits a significant genetic component. Neither tumour size at diagnosis, differentiation status nor tumour multiplicity were linked to BALB/c or CBA/Ca genotypes. This indicates that genetic factors influence mainly the early stages in tumour induction, whereas progression and clinical manifestation are probably governed by somatic alterations or epigenetic factors.

Except for the locus on chromosome 5, OS on different skeletal sites was equally affected by genetic predisposition. As expected from the smaller number of cases in every group, linkage to OS separated by tumour site was always weaker than to all OS combined. The only exception was the locus between D5Mit1and D5Mit34, for which OS of the vertebral column exhibit significant linkage (LRS = 14.1 or LOD = 3.06), whereas for all tumour sites combined linkage reached only suggestive value (LRS = 9.3). In general, bone tumours in the vertebral column are smaller at diagnosis, since they are usually detected at an earlier stage by the occurrence of clinically obvious paralysis. Their latency times, however, were not significantly different from the tumours at other parts of the skeleton. It is thus not clear why the locus on chromosome 5 has a more pronounced effect on this OS type.

Surprisingly, we found that at 2 of the 5 loci, the allele associated with higher OS risk were derived from the more resistant CBA/Ca strain. Similar observations, however, were already reported for other mouse strains and traits[11,14,30–32] and clearly challenges the assumption that all alleles derived from a susceptible mouse confer an increased risk in the progeny. Obviously, alleles with a latent effect upon tumour susceptibility might be present in the genome of a resistant strain and vice versa. It is also likely that some of those modifier alleles exert a phenotypic effect only in combination with other alleles.

To date human radiation susceptibility syndromes have been identified as monogenic and highly penetrant traits only (Ataxia Telangiectasia, OMIM 208900; Xeroderma Pigmentosum, OMIM 278700–278780; Nijmegen Breakage Syndrome, OMIM 251260; Nevoid Basal Cell Carcinoma Syndrome, OMIM 109400). They are caused either by defects in DNA repair genes or in genes involved in cellular signalling after radiation exposure. The high penetrance of these monogenic syndromes causes a mendelian segregation pattern, and thus facilitated positional cloning of the mutated genes. Spontaneous and therapy-related secondary OS, however, show evidence of congenital predisposition without a familial tumour aggregation.[8,33,34] This would either suggest polygenic effects and/or monogenic predisposition caused by inheritance of low-penetrance alleles.[35,36] The systematic search for allelic variants that govern cancer predisposition in man has just started. Mapping studies that make use of genetically defined laboratory animals, with their advantage of planned mating and reproducible tumour induction are certainly a valuable supplement for human association studies. The potential of animal studies to identify susceptibility genes and their allelic variants has already been proven

in several studies.[12,37,38] Some investigations could even show that human homologues of murine cancer susceptibility genes are associated with tumour susceptibility in man.[39,40] As outlined in the present study, animal studies to map tumour susceptibility loci might also be helpful to understand more general mechanisms of a congenital cancer predisposition.

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References

1. Gossner W, Hug O, Luz A, Muller WA. Experimental induction of bone tumors by short-lived bone-seeking radionuclides. Recent Results Canc Res 1976;54:36–49.
2. Rowland RE, Stehney AF, Lucas HF. Dose–response relationship for female radium dial workers. Radiat Res 1978;76:368–83.
3. Fry SA. Studies of U.S. radium dial workers: an epidemiological classic. Radiat Res 1986;150(Suppl):S21–9.
4. Spiess H, Mays CW. Bone cancers induced by 224 Ra (Th X) in children and adults. Health Phys 1970;19:713–29.
5. Tucker MA, D'Angio GJ, Boice JD, Jr, Strong LC, Li FP, Stovall M, Stone BJ, Green DM, Lombardi F, Newton W. Bone sarcomas linked to radiotherapy and chemotherapy in children. N Engl J Med 1987; 317:588–93.
6. Newton WA, Jr, Meadows AT, Shimada H, Bunin GR, Vawter GF. Bone sarcomas as second malignant neoplasms following childhood cancer. Cancer 1991;67:193–201.
7. Pitcher ME, Davidson TI, Fisher C, Thomas JM. Post irradiation sarcoma of soft tissue and bone. Eur J Surg Oncol 1994;20:53–56.
8. Hemminki K, Mutanen P. Genetic epidemiology of multistage carcinogenesis. Mutat Res 2001;473:11–21.
9. Balmain A, Nagase H. Cancer resistance genes in mice: models for the study of tumour modifiers. Trends Genet 1998;14:139–44.
10. Dragani TA, Manenti G, Della Porta G. Quantitative analysis of genetic susceptibility to liver and lung carcinogenesis in mice. Cancer Res 1991;51:6299–303.
11. Mock BA, Krall MM, Dosik JK. Genetic mapping of tumor susceptibility genes involved in mouse plasmacytomagenesis. Proc Natl Acad Sci USA 1993;90:9499–503.
12. Zhang SL, DuBois W, Ramsay ES, Bliskovski V, Morse HC, III, Taddesse-Heath L, Vass WC, DePinho RA, Mock BA. Efficiency alleles of the Pctr1 modifier locus for plasmacytoma susceptibility. Mol Cell Biol 2001;21:310–18.
13. Manenti G, Binelli G, Gariboldi M, Canzian F, De Gregorio L, Falvella FS, Dragani TA, Pierotti MA. Multiple loci affect genetic predisposition to hepatocarcinogenesis in mice. Genomics 1994;23:118–24.
14. Saito Y, Ochiai Y, Kodama Y, Tamura Y, Togashi T, Kosugi-Okano H, Miyazawa T, Wakabayashi Y, Hatakeyama K, Wakana S, Niwa O, Kominami R. Genetic loci controlling susceptibility to γ-ray-induced thymic lymphoma. Oncogene 2001;20:5243–47.
15. Ochiai Y, Tamura Y, Saito Y, Matsuki A, Wakabayashi Y, Aizawa Y, Niwa O, Kominami R. Mapping of genetic modifiers of thymic lymphoma development in p53-knockout mice. Oncogene 2003;22:1098–102.
16. Boulton E, Cole C, Knight A, Cleary H, Snowden R, Plumb M. Low-penetrance genetic susceptibility and resistance loci implicated in the relative risk for radiation-induced acute myeloid leukemia in mice. Blood 2003;101:2349–54.
17. Kodama Y, Yoshikai Y, Tamura Y, Wakana S, Takagi R, Niwa O, Kominami R. The D5Mit7 locus on mouse chromosome 5 provides resistance to γ-ray-induced but not *N*-methyl-*N*-nitrosourea-induced thymic lymphomas. Carcinogenesis 2004;25:143–48.
18. Rosemann M, Lintrop M, Favor J, Atkinson MJ. Bone tumorigenesis induced by α-particle radiation: mapping of genetic loci influencing predisposition in mice. Radiat Res 2002;157:426–34.
19. Muller WA. Studies on short-lived internal-emitters in mice and rats. II. 227 Th. Int J Radiat Biol Relat Stud Phys Chem Med 1971;20: 233–43.
20. Dietrich WF, Miller JC, Steen RG, Merchant M, Damron D, Nahf R, Gross A, Joycev DC, Wessel M, Dredge RD, Marquis A, Stein LD, et al. A genetic map of the mouse with 4006 simple sequence length polymorphisms. Nat Genet 1994;7:220–45. http://www-genome.wi.mit.edu/ftp/distribution/mouse_sslp_releases/may99/05-99.MarkerInfo.txt
21. Manly KF, Cudmore RH, Jr, Meer JM. Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 2001;12: 930–32.
22. Luz A, Erfle V, Rohmer H, Schetters H, Schafer E, Linzner U, Muller WA, Gossner W. [CBA- and Balb/c-mice: 2 inbred strains with different susceptibility for the induction of osteosarcoma by Thorium-227]. Verh Dtsch Ges Path 1982;66:516.
23. Luz A, Muller WA, Schaffer E, Murray AB, Linzner U, Gossner W. The sensitivity of female NMRI mice of different ages for osteosarcoma induction with 227thorium. Strahlentherapie [Sonderb] 1985; 80:178–82.
24. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995; 11:241–7.
25. Lloyd RD, Miller SC, Taylor GN, Bruenger FW, Jee WSS, Angus W. Relative effectiveness of 239Pu and some other internal emitters for bone cancer induction in beagles. Health Phys 1994;67:346–53.
26. Luz A, Muller WA, Linzner U, Strauss PG, Schmidt J, Muller K, Atkinson MJ, Murray AB, Gossner W, Erfle V, Hofler H. Bone tumor induction after incorporation of short-lived radionuclides. Radiat Environ Biophys 1991;30:225–7.
27. Mays CW, Spiess H. Bone sarcoma to man from 224Ra, 226Ra, and 239Pu. In: Muller WA, Evert HG, eds. Biological effects of 224Ra. The Hague: Martinus Nijhoff, 1978.168–81.
28. Ellenders M, Harrison JD, Pottinger H, Thomas JH. Induction of osteosarcoma and acute myeloid leukaemia in CBA/H mice by the α-emitting nuclides, uranium-233, plutonium-239 and americium-241. Int J Radiat Biol 2001;77:41–52.
29. Oghiso Y, Yamada Y. The specific induction of osteosarcomas in different mouse strains after injections of 239Pu citrate. J Radiat Res (Tokyo) 2003;44:125–32.
30. Tripodis N, Hart AA, Fijneman RJ, Demant P. Complexity of lung cancer modifiers: mapping of thirty genes and twenty-five interactions in half of the mouse genome. J Natl Cancer Inst 2001;93:1484–91.
31. Manenti G, Gariboldi M, Fiorino A, Zanesi N, Pierotti MA, Dragani TA. Genetic mapping of lung cancer modifier loci specifically affecting tumor initiation and progression. Cancer Res 1997;57:4164–6.
32. Le Voyer T, Lu Z, Babb J, Lifsted T, Williams M, Hunter K. An epistatic interaction controls the latency of a transgene-induced mammary tumor. Mamm Genome 2000;11:883–9.
33. Dong C, Hemminki K. Second primary neoplasms in 633,964 cancer patients in Sweden, 1958–1996. Int J Cancer 2001;93:155–61.
34. Hauben EI, Arends J, Vandenbroucke JP, van Asperen CJ, Van Marck E, Hogendoorn PC. Multiple primary malignancies in osteosarcoma patients. Incidence and predictive value of osteosarcoma subtype for cancer syndromes related with osteosarcoma. Eur J Hum Genet 2003; 11:611–18.
35. Fearon ER. Human cancer syndromes: clues to the origin and nature of cancer. Science 1997;278:1043–50.
36. Ponder BA. Inherited predisposition to cancer. Trends Genet 1990;6: 213–28.
37. Cormier RT, Hong KH, Halberg RB, Hawkins TL, Richardson P, Mulherkar R, Dove WF, Lander ES. Secretory phospholipase Pla2g2a confers resistance to intestinal tumorigenesis. Nat Genet 1997;17:88–91.
38. Manenti G, Peissel B, Gariboldi M, Falvella FS, Zaffaroni D, Allaria B, Pazzaglia S, Rebessi S, Covelli V, Saran A, Dragani TA. A cancer modifier role for parathyroid hormone-related protein. Oncogene 2000; 19:5324–28.
39. Dragani TA, Hirohashi S, Juji T, Kawajiri K, Kihara M, Ono-Kihara M, Manenti G, Nomoto T, Sugimura H, Genka K, Yokota J, Takahashi T, et al. Population-based mapping of pulmonary adenoma susceptibility 1 locus. Cancer Res 2000;60:5017–20.
40. Ewart-Toland A, Briassouli P, de Koning JP, Mao JH, Yuan J, Chan F, MacCarthy-Morrogh L, Ponder BA, Nagase H, Burn J, Ball S, Almeida M, et al. Identification of Stk6/STK15 as a candidate low-penetrance tumor-susceptibility gene in mouse and human. Nat Genet 2003;34:403–12.