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Search for a Genetic Link for Mammary Cancer in a Beagle Colony

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Abstract. *Background: A possible genetic link for malignant mammary tumor (MMT) was investigated. Materials and Methods: Records of an internally-irradiated beagle colony followed from the early 1950's until about 1995 were searched and analyzed by standard statistical procedures. Results: Only a single ancestor yielded a "p" value (Fisher's Exact Test) for an overrepresentation among descendants with MMT at <0.025 (one-sided test), and the number of comparisons for the 169 ancestors where the relative fraction of animals with MMT was greater than that for non MMT dogs (61) suggests that this could have occurred by chance alone. Results of other statistical tests were not remarkable. Conclusion: No genetic link for MMT in this colony could be established with the available data. These findings may or may not be relevant to humans.*

Between about 1950 and 1987, when the Utah beagle colony was moved to Albuquerque, NM, an experiment was conducted at the University of Utah that addressed the possible relative effects of various injected radionuclides on such endpoints as skeletal malignancies, soft tissue tumors and lifespan. Detailed clinical records in the archives of this moderately inbred, internally-irradiated beagle colony provided data that could prove to be useful in testing whether there could be established a familial inheritance for mammary cancer within this population, a study that may or may not have some bearing on mammary cancer in humans. Other investigators have published reports concerning a genetic link for malignant mammary tumors (MMT) in humans, dogs, rodents and other mammals (1-25). Rowland (26) reported an increased occurrence of MMT among human female radium workers, but was not able to determine whether this was a result of gamma radiation originating in the containers of radioactive paint used by these women (see photograph on page 20 of his book), the enhanced content of the radium decay product, radon (^{222}Rn), in the air within the facility, or the internal deposition of radioactivity in their bodies. Contained within the records of the Utah beagle colony were the location and specific histological type of all mammary tumors identified, dates of diagnosis of each MMT, birth date of each dog, injected radioactivity and the parentage of nearly every dog traced back through the generations within the colony to the AKC- (American Kennel Club) registered beagles from outside the project (founders). These founders had been parents of animals comprising the breeding colony (breeders) established within the laboratory. Reports of mammary tumor occurrence within the Utah beagle colony were published previously (27-31), but no analysis of the progenitors of dogs with or without MMT has been accomplished until now.

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Materials and Methods

Records of all female dogs in the Utah beagle colony given ^{226}Ra, ^{228}Ra, ^{228}Th, ^{90}Sr, ^{241}Am or monomeric ^{239}Pu as young adults by injection, or dogs which qualified as control animals not given radioactivity (27), were searched to discover which ones had ever been diagnosed with MMT (no MMT had been identified in any male dogs within these groups) (28). Because no MMT had been observed among any of these animals before about age 3 years (1,263 days, dog F012T30), dogs that did not attain this age were excluded.

Progenitors of each of the animals diagnosed with MMT at any time during their lifetimes and of each of the animals included in the study that were never diagnosed with MMT were compiled separately. Each appearance of a given progenitor within the pedigree of a dog was considered to be a "citation." Because the females given ^{226}Ra have been reported to have shown an excess of MMT as compared with the control females (27), they were analyzed separately from the other dogs in this study, in addition to their being included in the overall analysis.

For each of the comparisons, the number of citations for a given progenitor among the dogs with MMT and among the dogs without MMT were tabulated. Because of the importance of whether there might have been evidence of an overrepresentation of a given

Table I. *Results of statistical analysis of malignant mammary tumor (MMT) occurrence in first second-generation progeny of various ancestors compared with dog Mac85[a]. The dog chosen for comparison (Mac85) had an RF value (22 out of 64 = 0.344) near that of the mean RF for the entire group (136 out of 403 = 0.337) and a total of first second-generation progeny (64 dogs) that was near the mean for all dogs with number of progeny > 15 (60 dogs).*

	Number of progeny			
Dog	Progeny with MMT	Total progeny	RF[b]	*p* value[c]
Mac85	22	64	0.344	---
Jim	57	140	0.407	0.440
Fac53	3	11	0.273	0.742
Fac52	6	14	0.429	0.554
Binky	4	17	0.235	0.561
Spot	17	59	0.288	0.564
Ring	13	55	0.236	0.230
Queenie	9	24	0.375	0.806
Snooks	3	7	0.429	0.691
Bev	1	8	0.125	0.422
Mac84	1	4	0.250	1.00

[a]Mac and Fac were designations given to Male Aging Controls and Female Aging Controls, respectively.
[b]Relative Fraction = (MMT dogs)(total dogs)$^{-1}$.
[c]Probability that there is no significant difference from Mac85.

Table II. *Results of statistical analysis of malignant mammary tumor (MMT) occurrence in first second-generation progeny of various ancestors compared with dog Mac85.*

	Number of MMT per descendent (tumor dogs only)		
Dog	MMT[a](dog)$^{-1}$	Number of MMTdogs	*p* value[b]
Mac85	2.18±1.50	22	---
Jim	2.44±1.48	57	0.492
Fac53	2.00±1.73	3	0.848
Fac52	2.33±1.21	6	0.822
Binky	1.75±1.50	4	0.601
Spot	2.71±1.40	17	0.273
Ring	2.31±1.25	13	0.254
Queenie	3.44±1.51	9	0.042
Snooks	1.33±0.577	3	0.350
Bev	NA[c]		1
Mac84	NA[c]		1

[a]Mean±standard deviation.
[b]Probability that the mean is not significantly different from that of Mac85.
[c]Not suitable for analysis (only one dog with MMT).

ancestor among animals with MMT, only the instances in which the relative fraction [RF = (tumor dogs)(total dogs^{-1}] of a dog with descendants diagnosed with MMT was greater than that of the same animal whose descendants were not diagnosed with this disease were considered. In each case where the RF was greater for the MMT dogs, the data were analyzed by Fisher's Exact Test (InStat Instant Biostatistics, GraphPad Software, San Diego, CA, USA).

Since only the instances were considered where the RF was greater among dogs with MMT than among those without, a significance level of $p<0.025$ (one-sided test) was selected rather than the more conventional $p<0.05$ (two-sided test). It is well known, however, that for a data set in which 100 comparisons are made of non-significantly different pairs, something like 5 of them will indicate significance by chance alone at the 5% level (32) (p. 46) or about 1 out of 20. It follows, then, that at a significance level of 0.025, about 1 in 40 corresponding comparisons might indicate significance by chance alone.

Analyses of the first two generations descended from the most important ancestors (breeders) were made to determine whether an ancestor could be identified whose progeny were over-represented in dogs with MMT. The first two generations of all of the most prominent ancestors were analyzed by Fisher's Exact Test as compared with those of Mac85. These included Jim, Queenie, Ring, Spot, Fac52, Fac53, Mac84, Snooks, Binky and Bev. Excluded were ancestors with less than 10 descendants in the first two generations and those whose descendents had no MMT. The number of primary MMT per dog for each set of progeny was also compared to that of Mac85 by Fisher's Exact Test, supplemented by the Mann-Whitney non-parametric test for instances where the parametric test was not applicable. Another analysis included as a variable the age at diagnosis of each MMT, and a surrogate for the mammary radiation dose in the radium (^{226}Ra) dogs [the skeletal dose] was included as a variable, since the publication by Bruenger *et al.* (27) indicated that animals given ^{226}Ra appeared to have an enhanced probability of developing MMT. Analysis was accomplished by means of the BMDP asymptotic covariance matrix (accelerated failure time model) (BMDP Statistical Software, Inc., Los Angeles, CA, USA). The set of progeny of each progenitor was compared with that of Mac85 and, in another analysis, was compared with the female control dogs (of variable ancestry).

A description of animal selection, study design, animal husbandry, necropsy procedures, histological evaluation of tissues, *etc.* can be found in previous publications from this laboratory (33-36). The beagle colony was maintained in compliance with contemporary standards for laboratory animal management as mandated by the University of Utah Animal Use and Care Committee, the U.S. Department of Agriculture and the American Association for Accreditation of Laboratory Animal Care. Anesthesia and/or analgesia were used prior to any procedure that might cause undue pain or discomfort. Nearly all the beagles used in the lifespan study were produced by the on-site breeding colony. A few (5), obtained directly from commercial breeders, were included. The breeding stock was obtained from commercial breeding kennels, and virtually all of these animals were offspring of AKC- registered (champion) beagles.

Results

The female dogs in the study who were given ^{226}Ra and were diagnosed with MMT totaled 20, and those without MMT totaled 39 (female beagles given this radionuclide have an

Table III. *Results of statistical analysis of malignant mammary tumor (MMT) occurrence in first second-generation progeny of various ancestors compared with dog Mac85.*

Number of MMT per descendent (all progeny)			
Dog	MMT[a](dog)$^{-1}$	Number of dogs	*p* value[b]
Mac85	0.750±1.36	64	---
Jim	0.993±1.52	140	0.276
Fac53	0.545 ±1.21	11	0.641
Fac52	1.00±1.41	14	0.537
Binky	0.412±1.00	17	0.341
Spot	0.780±1.44	59	0.118
Ring	0.545±1.15	55	0.382
Queenie	1.29±1.92	24	0.524[c]
Snooks	0.571±0.787	7	0.734
Bev	0.125±0.354	8	0.277[c]
Mac84	0.75±1.50	4	1.00

[a]Mean±standard deviation.
[b]Probability that the mean is not significantly different from that of Mac85.
[c]Results of Mann-Whitney non-parametric analysis, since *p* values for differences in standard deviations for the individual dog and Mac85 were < 0.05, and so the data sets were not suitable for parametric analysis.

elevated occurrence of mammary cancer as compared with control females) (27). These animals had 94 ancestors. For 53 of these progenitors, the relative fraction (RF) of the MMT dogs was less than in the non MMT dogs, and for 41 of them, the RF of the MMT dogs was greater than in the non MMT dogs. The *p* values of these comparisons ranged between 1.0 and 0.053. There was none <0.025. Therefore, the dogs given ^{226}Ra were included with those given other radionuclides and with the control animals, since the results for ^{226}Ra dogs in this analysis were unremarkable.

When all female dogs in the study were analyzed together, it was found that 125 dogs had MMT and 283 had no MMT. Also, their ancestors totaled 169. There were 108 where the RF of dogs with MMT was less than the RF of dogs without MMT, and 61 ancestors had an RF for dogs with MMT that was greater than for those without MMT. Except for a single ancestor with a *p* value of <0.025 (Goldy Fac16, MMT = 8 of 125 MMT or 0.064 *vs* 2 of 283 non MMT or 0.0071), *p* values ranged between 1.0 and 0.088. The single *p* value <0.025 was based upon only 10 out of 408 citations and might well have been a result of chance alone.

Analysis of progeny with MMT *vs* those without MMT (Tables I-III) as compared with those of dog Mac85 (RF = 0.344) revealed that *p* values from Fisher's Exact Test ranged from 0.230 to 1.00 (Table I). When only the progeny with MMT were considered for the number of MMT per dog (Table II), *p* values when compared with Mac85 ranged between 0.042 (Queenie) and 0.848; with all progeny included (with and without MMT, Table III), *p* values for the number of MMT ranged between 0.118 and 1.00 (two of these 10 comparisons were by Mann-Whitney methods because the data were not suitable for parametric analysis). With the exception of only 9 MMT progeny of Queenie (Table II), none of these comparisons was statistically significant.

The results of the accelerated failure time model (corrected for mammary radiation dose from ^{226}Ra) indicated that no significant differences in survival to MMT diagnosis from progeny of Mac85 (61 first- and second-generation progeny) could be detected except for lower values for Fac52 (14) and Spot (51). This analysis indicated that there was no significance to any differences to time of MMT diagnosis with respect to progeny of Mac85 for progeny of Queenie (24 descendants), Jim (126), Ring (51) or Fac53 (11). There seemed to be no consistent pattern with respect to the other analyses performed as far as the progeny of Fac52 and Spot were concerned.

When the first second-generation progeny of the progenitors were compared to the survival of the control females by BMDP, it was shown that for 4 of the ancestors (Fac53, Queenie, Mac85 and Ring), controlled for the contribution of radiation dose, the lifetimes were less than those of the controls, but the differences from controls were not significant. Survival of the first two generations of the other progenitors (Jim, Fac52 and Spot) was significantly greater than the controls. Therefore, life shortening as a result of mammary cancer was not evident.

Analysis by Fisher's Exact Test of the age at death (Table IV) for the first second-generation progeny of these ancestors, compared with those of Mac85, indicated that no significant differences could be established. However, when the age at death for all of the first second-generation progeny were analyzed, 5 of the 10 progeny sets were significantly different from Mac85. However, in 9 of the 11 data sets, the means for all progeny were less than the means for the MMT dogs only. This might indicate that dogs without MMT had substantially shorter lifetimes than the dogs with MMT. This is demonstrated in the final two columns of Table IV. Seven of the 10 means were significantly less than that of Mac85, and for only 2 dogs was the non MMT mean greater than the MMT mean (Mac85 and Fac53).

Discussion and Conclusion

These analyses appear not to support an important genetic link for MMT among female dogs in the Utah beagle colony, so such an effect should not be included in analyses of skeletal or other malignancy. Since humans have only two mammae and dogs have about eight, some of the findings about our beagles may not be directly applicable to humans.

Table IV. *Results of statistical analysis of malignant mammary tumor (MMT) occurrence in first second-generation progeny of various ancestors compared with dog Mac85.*

Mean age at death (years)						
Dog	MMT dogs[a]	*p* value[b]	All dogs[a]	*p* value[b]	Non MMT dogs[a]	*p* value[b]
Mac85	11.6±2.4	----	12.2±2.5	----	12.5±2.5	----
Jim	11.7±3.0	0.880	9.3±3.9	<0.0001[c]	8.1±3.7	<0.0001[c]
Fac53	11.8±1.0	0.884	13.4±2.0	0.140	14.0±2.0	0.128
Fac52	13.0±1.3	0.976	11.3±3.4	0.634[c]	10.0±4.0	0.149[c]
Binky	10.1±4.4	0.918[c]	7.8±3.4	<0.0001[c]	7.0±2.9	<0.0001
Spot	12.6±2.7	0.197	9.4±3.7	<0.0001[c]	7.9±3.1	<0.0001
Ring	12.8±2.0	0.119	9.4±3.7	<0.0001[c]	8.3±3.5	<0.0001[c]
Queenie	13.4±2.3	0.068	10.8±3.9	0.251[c]	9.3±4.0	0.0071[c]
Snooks	11.6±4.1	0.976	10.0±3.8	0.156[c]	8.9±3.6	0.0105
Bev	11.6[d]	NA	8.2±3.8	0.0082[c]	7.7±3.8	0.0049[c]
Mac84	12.7[d]	NA	11.4±1.6	0.472	11.0±1.6	0.261

[a]Mean ± standard deviation.
[b]Probability that the mean is not significantly different from that of Mac85.
[c]Results of Mann-Whitney non-parametric analysis, since *p* values for differences in standard deviations for the individual dog and Mac85 were < 0.05, and so the data sets were not suitable for parametric analysis.
[d]Not suitable for analysis (only one dog with MMT).

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