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RADIATION-INDUCED DOMINANT SKELETAL MUTATIONS IN MICE: MUTATION RATE, CHARACTERISTICS, AND USEFULNESS IN ESTIMATING GENETIC HAZARD TO HUMANS FROM RADIATION*

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INTRODUCTION

Over the last three decades, especially, a wealth of information has been gathered on the effects of radiation in inducing mutations. The impetus, of course, has largely been to improve our understanding of the amount of genetic hazard to mankind from radiation exposures so that wise policy decisions could be made for regulating exposure limits. Curiously, one of the biggest difficulties faced in this search for knowledge has been trying to unravel the extent to which an increase in the mutation rate would be expected to increase the load of genetic disorders in the human population. For the most part, experiments designed with the goal of quantifying deleterious effects on populations, often following many generations of high-dose exposures, had results which were either negative or extremely difficult to relate to public health. Green [1], in reviewing a large number of such studies in 1968, cautioned, however, that the generally negative results of such studies might be due not to the nonexistence of induced mutations having only moderate individual effects in heterozygotes but possibly to the failure to have found good enough indicator traits or to the relatively small sizes of the experiments that had been done and their relative lack of power for discriminating small genetic differences in the presence of large amounts of nongenetic variability. Even as recently as 1972, understanding of the amount of induced dominant damage in mammals was so poor that the UNSCEAR Report of that year [2] had to make use of the estimate of the fraction of induced recessive mutations in Drosophila that adversely affects fitness of heterozygotes in terms of fertility, viability, and so on in calculating its risk estimate. The work that will be discussed in this paper represents a major advance in the difficult task of trying to estimate the effects that an increase in the mutation frequency would have upon human health.

A series of experiments by Ehling [3-5] laid the groundwork for the experiments to be described. In the mid-1960's he attempted to measure empirically part of the overall induced damage to the body by determining the frequency of induction of dominant mutations that cause malformations in the skeletons of mice. The skeleton was chosen for this attempt to measure overall damage to one body system largely because it can be prepared readily for detailed study. Dominant mutations, which could of course be detected in the first-generation following irradiation, were of special interest because they are the type of mutation that would account for almost all induced damage in the early generations. Ehling developed criteria based upon the degree of rarity with which anomalies occurred as the means of distinguishing variants caused by dominant mutations from those of non-mutational origin, but his procedures permitted no verification that suspected mutations were really mutations by means of breeding tests. His experiments, using a number of different

radiation exposures and a control, suggested strongly that large-dose irradiation induced a high frequency of dominant mutations causing skeletal malformations. However, probably because there was no clear proof that his presumed dominant mutations were mutations, his data were never used in estimating overall risk by committees, although Russell [6] states that he always "felt that a mutation rate based on these presumed mutations would probably not be in serious error." Before discontinuing research on skeletal mutations, Ehling did find three dominant skeletal mutations that were transmitted to later generations [7].

MUTATION-RATE EXPERIMENT

The procedures and results, published in detail elsewhere [8-10], will be briefly summarized. Our experiment was performed in Ehling's Division in the Gesellschaft für Strahlen- und Umweltforschung MBH in Neuherberg, Germany, using sublines of the same strains of mice used by Ehling in his earlier skeletal experiments in the Biology Division of Oak Ridge National Laboratory. Male mice were exposed to 100 R + 500 R of cesium-137 gamma radiation (60 R/min) with a 24-hour interval between fractions, this dose being chosen because it had yielded the highest mutation rate in Ehling's experiments. Only male offspring derived from spermatogonial irradiation were used. Unlike Ehling's experiments in which the F_1 progeny were prepared for examination at about four weeks of age, the males in our experiment were not killed for skeletal preparation until they had been given a chance to breed with at least three females each. F_1 males were then killed, cleared and alizarin-stained skeleton preparations were made, and these were studied in detail under a dissecting microscope. If the male had skeletal abnormalities that suggested that he might possibly be a mutant, some of his offspring could usually be killed and examined to determine whether the abnormalities were transmitted. By means of this breeding test, mutations could be confirmed. Usually a mutant line could be established from the remainder of a mutant's progeny. No concurrent control was studied since we had decided to concentrate our efforts on examining a large irradiation sample. Furthermore, Ehling's earlier control experiment made it seem certain that very few mutations would be found in a control sample. In order not to overlook mutations by using criteria that were too restrictive in deciding which F_1's required a check of the next generation, guidelines for deciding which F_1's should be tested were made very broad. As a result, offspring of 228 F_1's were examined even though for the great majority of these it would have been surprising had they been found to be mutants.

Thirty-seven dominant mutations were found in the sample of 2646 F_1's. This is a mutation frequency of 1.4% per gamete. Thirty-one of the F_1's were confirmed to be mutants by means of the breeding tests [9], and six, having no progeny, were considered mutants on the basis of presumed-mutation criteria supported by the data [10]. Because it seems certain that the spontaneous frequency must be very small in comparison to the induced frequency, and because it is certain that some mutations were overlooked as the result of viability effects and incomplete penetrance, we have assumed that 1.4% is a reasonable estimate of the induced frequency, with any included spontaneous mutations probably being at least counterbalanced by mutations that were overlooked. For a number of reasons [8], it seems certain that very few, if any, of the mutations were present in the stocks before the experiment was started.

GENERAL DESCRIPTION OF DOMINANT SKELETAL MUTATIONS

Almost all skeletal effects were (1) fusions of bones or other changes in the number of individual bones, (2) gross changes in bone shape, usually caused by

incomplete or too extensive bone growth, or (3) shifts in the relative position of bones. For three of the mutations, the only skeletal effect was a marked reduction in body size. Almost all regions of the skeleton were affected by at least one of the mutations, and most mutations caused multiple effects. To illustrate this, when specific anomalies were counted, it was seen that five of the 31 proved mutations caused one anomaly each, 19 caused 2-5 anomalies, five caused 6-10 anomalies, and two caused 11-13 anomalies. Many parts of the mouse skeleton are strikingly similar to the human skeleton, and a great many of the malformations are quite similar or identical to those seen in various human genetic disorders. One entire syndrome, which occurred twice in independent mutations, has been recognized as having a strong resemblance to the syndrome in humans known as cleidocranial dysplasia.

On the basis of the 31 proved mutations, it seems to be a valid generalization that dominant mutations have incomplete penetrance for some or all of their effects. At least nine of the 31 proved mutations have incomplete penetrance for every effect that they are known to cause. One interesting example is provided by one of the mutations which is capable of subdividing the interparietal bone in the skull. In 17% of the carriers of this mutation, this bone is completely normal, but in 55%, 24%, 3%, and 1% it is divided into 2, 3, 4, and 5 pieces, respectively. It is clear that mice can be greatly deformed without showing any sign of this externally. In fact, most of the mutations cause no effect that is externally visible, and of those that do cause an externally visible effect, most of these effects occur in only a small proportion of carriers.

MANY DOMINANT MUTATIONS ARE ALSO RECESSIVE LETHALS.

Following the mutation-rate experiment, I assumed my present position at Oak Ridge National Laboratory. Mutant lines for many of the mutations were successfully transferred to my new location. One question of interest in studying these was whether or not many of them were recessive lethals. One reason why this was of interest was that a majority of specific-locus mutations, the type of mutation that we know most about in mammals, are recessive lethals. Would this also hold true for dominant skeletal mutations? Another reason this question was of interest was because of claims in the literature [11-12] that recessive lethals generally have no deleterious effects in heterozygotes. There clearly was no question but that many of the dominant skeletal mutations had deleterious effects. Yet a third reason for interest was that homozygous lethality would suggest that a given mutation was likely to be a small deficiency. We have already reported that the first four dominant skeletal mutations tested for homozygous lethality were found to be lethals acting between implantation and late pregnancy [13]. These studies have now progressed to the point where we know that at least seven of the first eight dominant skeletal mutations set up for testing are recessive lethals acting between implantation and birth.

For the mutations having full penetrance for at least some of their effects, the test for homozygous lethality is straightforward and easy. Proved carriers for mutations are outcrossed to non-carrier females and the offspring are collected. The offspring are mated at random, avoiding full-sib crosses. Uterine examinations are made in late pregnancy, at which time both parents are killed and prepared for skeletal examination. Because the skeleton examinations reveal the genotypes of all mice, the results of the uterine examinations can easily be matched up with the different crosses of genotypes with complete accuracy. Recessive lethality in the period between implantation and birth can be recognized by the expected higher death rate of implants in the approximately one-quarter of the crosses that are between heterozygotes for a given mutation. Data for mutation number 320, one of those with the syndrome similar to cleidocranial dysplasia, illustrate this. For the crosses +/+ X +/+, 320/+ female X +/+ male, +/+ female X 320/+ male, and

320/+ X 320/+, the proportions of dead implants were 19/207 (or 9.2%), 20/217 (or 9.2%), 24/229 (or 10.5%), and 73/198 (or 36.9%), respectively. The spontaneous frequency of dead implants in these mice is about 10.6%, which means that a recessive lethal mutation killing exclusively between implantation and late pregnancy should cause about 33.0% dead implants in the intercross of carriers. Clearly, mutation 320 fits this model well. All pertinent statistical comparisons support the conclusion that it is a recessive lethal.

Testing for homozygous lethality is more difficult if a mutation has incomplete penetrance for all of its effects. In this case some of the +/+ X M/+ crosses (M = dominant skeletal mutation) and possibly even some of the +/+ X +/+ crosses are really M/+ X M/+. Again, the death rate in the intercross of carriers would be expected to be about 33.0% for recessive lethals acting during this time frame; however, the death rates in the crosses of heterozygotes by supposed non-carriers may be considerably elevated by contamination with M/+ X M/+ crosses, thus raising the question of whether the high intrauterine death rate in the intercross of carriers results from recessive lethality or, instead, from dominant lethality in which the homozygote is no more easily killed than the heterozygote. The question of whether the mutation causes some dominant lethality, and if so how much, can be easily resolved by determining the frequency of dead implants in crosses of known heterozygous males to non-carrier females. If no evidence of dominant lethality is found, it can be concluded that the reason for the high intrauterine death rate in the intercross of carriers is indeed homozygous lethality. The following data illustrate a case in which it was necessary to test for dominant lethality in order to be sure a mutation was a recessive lethal. Intrauterine death rates for mutation 1629, which has incomplete penetrance for all of its effects, were 31/189 (or 16.4%) in the cross +/+ X +/+, 24/86 (or 27.9%) in the cross 1629/+ female X +/+ male, 19/91 (or 20.9%) in the cross +/+ female X 1629/+ male, and 24/51 (or 47.1%) in the cross 1629/+ X 1629/+. The follow-up experiment in which known heterozygous males were mated to +/+ females showed, surprisingly, that mutation 1629 has incomplete penetrance for dominant lethality. Intrauterine death was 35/166 (or 21.1%), which is significantly higher than the death rate in all known +/+ X +/+ crosses in our laboratory (which is 58/546 or 10.6%), $P < 0.0003$ in a one-tailed test. The point estimate of dominant lethality is that 23.5% of M/+ implants die before late pregnancy. Expected death in the intercross of heterozygotes, with mutation 1629 being both a recessive lethal and having the stated degree of dominant lethality, is 43.4%. The observed frequency of 47.1%, which does not fit the model based on mutation 1629 being a recessive lethal alone, fits this model well.

One important conclusion from these results is that recessive lethal mutations probably account for an important fraction of human genetic disorders owing to their dominant deleterious effects. This seems certain when one considers the following results of homozygous lethality testing carried out on the two groups of dominants that have been most extensively tested. Of all possible dominant mutations, dominant visibles represent only a very small fraction, but, because of their easy detection, they have been studied more than other dominants. At least 45, or 27%, of 164 dominant visibles in the mouse (ignoring those concerned with enzyme polymorphisms and immunological traits) appear to be recessive lethals [14]. The dominant skeletal mutations, however, are part of that great majority of dominant mutations that are not externally visible. The finding of at least seven recessive lethals out of eight tested from among this huge category of dominants shows that frequent homozygous lethality is by no means peculiar to dominant visibles (and specific-locus mutations). Sheridan [15] argued that generalizations about dominant deleterious effects of recessive lethals cannot be drawn from tests done on dominant visibles because such tests are biased. Since the dominant skeletal mutations were discovered because they cause morphological damage, tests to see if they are recessive lethals are also obviously biased in the direction of showing that

recessive lethals have dominant deleterious effects. However, in view of the relative mutation frequencies for dominant skeletal mutations and recessive lethal mutations [16] and the high proportion of dominant skeletal mutations that causes homozygous lethality during the same period of development studied by Lüning and his co-workers [11, 12, 16], it is apparent that a great number of recessive lethals with important dominant deleterious effects occurred in the experiments on recessive lethals performed by Lüning and his co-workers. Even though they could have recognized these mutations as recessive lethals, the important dominant effects of these mutations would have been undetected by their procedures. It is apparent that recessive lethals often have dominant deleterious effects.

Interestingly, of the eight mutations set up for testing, two are known to have incomplete penetrance for low levels of dominant lethality, two others are suspected of having such, and for one we have little data at this time. Thus, the conclusion of Lüning and Sheridan [11-12] that recessive lethals generally lack dominant lethal effects perhaps needs to be reexamined also. As these authors pointed out, many of the recessive lethals that they studied were drawn from later generations of experiments in which there were repeated irradiations over many generations, and therefore the mutations that they studied might possibly be a somewhat atypical sample of recessive lethals.

A FEW DOMINANT SKELETAL MUTATIONS ARE ALSO BALANCED TRANSLOCATIONS.

It has been dogma in human genetics that balanced translocations themselves impart no deleterious effects to the carriers but only to their occasional offspring who are segmental aneuploids [17]. Recent risk estimates [14, 18] for radiation-induced chromosomal aberrations continue to be made only for unbalanced zygotes, although as will be shown later this leads to no underestimate of risk.

It is now known that at least three of the 37 dominant skeletal mutations are reciprocal translocations, that is, the mice have malformations because they are heterozygous for a reciprocal translocation. We have recently demonstrated this by showing that the frequency of induction of heritable translocations [19] is too low to explain, on the basis of chance, the occurrence of an independent translocation and dominant skeletal mutation within about 10 cM of each other in a sample of 37 dominant skeletal mutations. The mapping was done by looking for the association of the mutation with the presence of multivalents in metaphase I preparations of spermatocytes of males having about a 50% chance of carrying a mutation. All three mutations showed a perfect correlation. The results of this experiment suggest further that most radiation-induced heritable translocations in mice cause malformations. The point estimate is that about one-half of induced reciprocal translocations cause malformations that would be a serious handicap if they occurred in humans, but the confidence limits are very wide. Nonetheless, these data make it seem likely that radiation-induced translocations harm more balanced individuals than unbalanced ones.

Perhaps these findings will eventually lead to the recognition of similarly acting translocations in humans. In this regard, it is interesting to note that of 77 human infants with balanced rearrangements reported from newborn series, seven manifested either more than one congenital anomaly or poor growth [20]. It is known [20] that de novo balanced autosomal rearrangements are much more prevalent among mentally retarded individuals; however, the three skeletal mutations that are reciprocal translocations, which were induced in our experiment, have been shown to be transmissible.

METHODS OF APPLYING THESE DATA IN ESTIMATING FIRST-GENERATION RISK IN 1977 UNSCEAR AND 1979 BEIR REPORTS

The most important features of the dominant skeletal mutation-rate data that make them useful in estimating risk are that they provide both an estimate of the induced mutation frequency for dominant mutations causing abnormalities in one system of the body and a detailed description of the abnormalities so that they can be judged as to their importance. Although the skeletal approach is in an early stage of development, these results have already provided a basis for major new approaches in risk estimation adopted in the 1977 UNSCEAR Report [14] and in the recently released 1979 BEIR Report [18]. Both reports estimated genetic risk for both the first generation and for genetic equilibrium. As of yet, the skeletal approach has not been extended beyond the first generation, so it can only be used to estimate first-generation risk. The ways in which these estimates were made will be shown. In both cases the estimates will be given for increased exposure to 1 R (or rem) of X-rays or gamma rays over a period of 30 years.

Both Committees initially converted the mutation frequency reported to that expected in spermatogonia under the exposure conditions of 1 R of protracted irradiation. This was done by dividing the mutation frequency of 37/2646 by 600 R to correct for the dose, by 3 to correct for the dose-rate effect, and by 1.9 to correct for the fractionation effect. The last two corrections are based on the assumption that kinetics of induction are similar to those of specific-locus mutations, for which these corrections apply. This yields a mutation frequency of 4×10^{-6} for all dominant skeletal mutations.

The UNSCEAR Committee used the best-estimate approach of estimating risk. In order to estimate risk based on the induced frequency of dominant skeletal mutations, it was necessary to expand this frequency to all body systems and to restrict it to those mutations having serious effects. This was done by the UNSCEAR Committee in the following way [14]. On the basis of McKusick's tabulation of monogenic disorders in humans, it was determined that 74 (or roughly one-fifth) of the 328 clinically important disorders involved one or more parts of the skeleton. This would suggest that you might have to multiply the number of skeletal mutations by five to get the total number of dominant mutations. However, this fraction of one-fifth is likely to be too high as a result of the ease of diagnosis of skeletal defects by phenotypic inspection, radiography, or both. It seemed unlikely that this fraction would be exceedingly much lower, however, since it is well known in humans and mice that many dominant mutations show pleiotropism and affect more than one body system. In view of these considerations and the opinions of the noted human geneticists Carter and McKusick, it was decided that the true figure was likely to be of the order of 10%, in which case a factor of 10 would be used to expand the mutation frequency for dominant skeletal mutations to all body systems. It was also clear that many of the dominant skeletal mutations have no known effects that would be a handicap if they occurred in humans. The main reason for concluding that about one-half of the mutations would lead to a serious handicap in humans was that this figure seemed reasonable in view of a detailed discussion of the 37 individual mutations by McKusick and me, at the request of the Committee.

As stated, it was decided that dominant skeletal mutations would probably represent about 10% of total dominant mutations and that about one-half of the mutations cause effects that would impose a serious handicap if they occurred in humans. Multiplication of the mutation frequency of 4×10^{-6} by 10 and then by $\frac{1}{2}$ yielded a risk estimate of 20 induced serious dominant disorders per million liveborn offspring following increased paternal irradiation of 1 R per generation of 30 years. No estimate was made for maternal irradiation, but it was assumed that risk would be low in comparison to that of the male. The UNSCEAR Committee made a second estimate of first-generation risk from dominant disorders by using

the very different doubling-dose method, which is not based on an estimate of induced damage. The two estimates were in close agreement.

The BEIR Committee based its first-generation estimate on the skeletal data alone, although it did mention that a doubling-dose estimate would be in reasonably good agreement. In contrast to the UNSCEAR Committee, it estimated risk using the range-estimate approach and it included maternal risk in its calculations. It assumed that from 1/4 to 3/4 of the dominant skeletal mutations have serious effects and that the total number of dominant mutations would be adequately approximated by multiplying the skeletal mutation frequency by a range of from 5 to 15. The resultant risk estimate was 5 to 45 serious induced dominant disorders per million liveborn progeny for paternal irradiation. Based on specific-locus data, it concluded that maternal risk would be anything from negligible to 44% of that in the male. Accordingly, the final risk estimate was 5-65 induced dominant disorders per million liveborn humans.

ADDITIONAL DISCUSSION

There are many ways in which the skeletal approach for estimating risk can be refined. Although this approach for estimating risk is independent of the many problems associated with determining the current incidences of human disorders [21-22], which must be known for calculating risk by the doubling-dose method, increases in knowledge in the area of human genetics will nonetheless improve risk estimates made using the skeletal data. It seems reasonable to expect that some studies on the genetic nature of dominant skeletal mutations, for example the experiment mentioned on translocations, will prompt some of the hoped for advances in human genetics.

Incidentally, the fact that the BEIR and UNSCEAR Committees made no risk estimate for balanced translocations, when they estimated risk for chromosomal aberrations, led to no underestimate of risk because those translocations that mimic dominant mutations were already included in their estimate for induced dominant disorders that was based on the skeletal data.

One especially important application of the skeletal approach will be to extend estimates of risk based on induced damage to later generations. Some of the possible ways of doing this have been described [23]. Even though 6 of the 37 dominant skeletal mutants were possibly sterile, fertility of carriers for most of the mutations is not noticeably reduced. For this reason, there is no reason to doubt that there will be an accumulation of skeletal malformations in the population when multigeneration experiments are performed. It will be of considerable importance, however, to discover the rate and extent of accumulation of those mutations that cause serious effects. As Green [1] suspected might be the case, it now seems certain that the reason for the generally negative results in earlier population studies was that the great majority of those experiments dealt with components of fitness or other traits that show so much normal variation that moderate or small increases in deleterious effects could go undetected. Of course, many important genetic disorders may not affect reproductive fitness much anyway [21].

The approach, initiated by Ehling and strongly encouraged by W. L. Russell [6, 24] of empirically measuring part of the overall induced damage by identifying as much induced damage as possible in the skeleton of the mouse shows promise of greatly improving estimates of risk from radiation and other mutagens.

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