Emerging perspectives in radiation genetic risk estimation

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The farewell lecture delivered by Prof. K. Sankaranarayanan, Department of Radiation Genetics and Chemical Mutagenesis, Leiden University Medical Centre on October 23, 1998 during the International Symposium on "Somatic and genetic risk of ionising radiation: emerging perspectives" organized in his honour.

Universiteit Leiden

Colleagues and friends,

Society dictates that towards the end of a professional career, when an individual reaches a certain chronological age, which varies with the nations of the world, he or she steps down from his or her position to make room for younger people. For professors at Leiden University, the tradition has been that they give an inaugural lecture at the start of their professorial career at which time they try to project a vision of what they hope to achieve and a farewell lecture when the term of office comes to an end. The latter occasion is used to look back, from a personal perspective, at the events that dotted their life's calendar, reminisce over the persons and personalities who have had a major impact on their career, reflect on the extent to which the vision projected at the beginning was fulfilled, and present some views on what the future may hold for the field they came to be identified with.

When I look back at the last 33 years I have spent in Leiden, roughly thirds of this period as a professor, I feel a great sense of satisfaction and pleasure. In fact I feel blessed. I came to Leiden on 11th November 1965 from The Rockefeller University, New York, where I was doing my post- doctoral work with the now late Prof. Dobzhansky, one the most famous population geneticists and one of the most powerful intellects of this century with whom I had the privilege of doing my Ph.D at Columbia University, New York, when he was still there. He instilled in me a sense of how to do science, one which was to prove very important in all my career. My scientific work in the United States was on assessing the adverse genetic consequences of radiation exposure in populations of fruit flies, namely, Drosophila, a very favorite experimental organism then as well as now to study this and several different kinds of problems. Little did I know at that time that I am going to devote a major part of my scientific career to the study of genetic consequences of radiation exposure in humans.

The now late Professor Sobels, whom we called Frits and who headed our department here till 1987 was instrumental in my coming to Leiden in 1965. I did not know him or his work at that time or that he was one of the greatest scientists in Europe. I first saw his name as

editor of a new Journal called Mutation Research in 1964 at the Rockefeller University library. Since I had already decided to learn something more in addition to population genetics in some other laboratory in the world, the field of mutation research appealed to me. Further, from the papers he published in the first few issues of Mutation Research, I knew he was also a Drosophila geneticist. So, I corresponded with him, and within three weeks, I was offered a position as a senior scientific officer. In his letter, he noted that my appointment was for a year and if I agreed to stay for at least two years, he could pay the travel expenses for me and my family, one half when I came and one half when I left. In 1968, the situation changed: he told me that he would like me to stay permanently here and help him along with the other colleagues, to build the department and in editing Mutation Research. I feel so fortunate that I could never collect the second half of my travel expenses !

Our relationship transcended the purely professional and the bonds of friendship and mutual respect became stronger and stronger with time. I mention all these because he was the second most important person in my career. Frits Sobels was a scientist with vision and he had one simple philosophy: if you want a build a department, be open to ideas, get the right kind of people, give them the freedom and a carefree environment to pursue and sustain their scientific vision, watch them grow and derive a sense of shared delight in their accomplishments. Paul Lohman who succeeded Frits Sobels not only ensured that what Frits Sobels built up endured but strengthened it and built on it in more ways than one.

Frits Sobels applied the same philosophy also to the Journal, Mutation Research which he founded in 1964 and which has grown by leaps and bounds to become a premier Journal in this ever diversifying field. A couple of months ago, in its 34th year of existence, we published the 400th volume of the Journal. I am proud to be still one of its editors and this journal remains a part of my life. We grew up together in Leiden.

The next major change in my professional life — one that helped to define my rsearch career for which I am known, came in 1970

when Frits Sobels recommended my name to be a consultant in genetics to the United Nations Scientific Committee on the Effects of Atomic Radiation abbreviated as UNSCEAR, a scientific body which now representss 21 member states. His recommendation was readily accepted by the then Scientific Secretary of the Committee, Dr. Francesco Sella, an Italian, who had great confidence in his judgement. In taking up this assignment, I had the obligation to prove worthy of their collective confidence in me and do what was expected. The latter was to critically appraise and effect a synthesis, in the form of a comprehensive report, of all data in organisms ranging from bacteria to humans on genetic effects of radiation, draw conclusions on their relevance to the estimation of genetic hazards of radiation exposure of humans, which the Committee can use for its deliberations. These reports were subsequently published by the United Nations as official documents and are considered the most authoritative documents on the subject. I have the honor and privilege of serving this Committee since 1970 and being responsible for genetics in the 1972, 1977, 1982, 1986, 1988 and 1993 reports. I am now busy with the millenium report to be published in the year 2000.

Judging from the rapid advances occurring in the field of human genetics with which genetic risk estimation is intimately linked, and the need to keep abreast of how these are used in risk estimation, in 1997, the Committee took the step, unprecedented in its 40 year history, of inviting me, a consultant, to address the whole committee in a 45 minute plenary lecture on advances in genetic risk estimation. I had the honor of doing a similar lecture to the Committee, again in 1998 and one further lecture is scheduled for 1999. The important dividends of UNSCEAR work, however, are the opportunities to develop a broader perspective of the whole field of radiation research and get to know the scientists from all over the world both within and outside the framework of UNSCEAR. I still cherish the opportunities this work had engendered, namely, to visit the laboratories and discuss science with a number of giants and pioneers who have made phenomenal contributions to the field of radiation genetics and risk estimation. These include, Bill and Lee Russell in Oak Ridge, Jim Neel in Ann Arbor,

K.G. Luning in Sweden, Udo Ehling in Munich, Mary Lyon and Tony Searle in the United Kingdom and several others. Friednships with most of these people have continued to the present.

So, in the mid-1970s, when I was elected to serve as a member of the Radiobiology Committee of the International Commission on Radiological Protection or ICRP, it was an other important milestone. ICRP is another major international organization engaged not only in the analysis of data on radiation effects, but also in formulating recommendations and guidelines for the protection of man from the hazards of radiation exposure which are used all over the world. This gave me an unique opportunity not only to expand my horizons further but also to look at my science from a different perspective and make new friends in the fields of radiation epidemiology, radiation dosimetry and radiation protection from all over the world. It was here I had the pleasure of really getting to know Jack Schull from the University of Texas who along with Jim Neel and several Japanese colleagues have done the seminal and most comprehensive of studies on radiation effects in humans ever done, namely, on the populations of Hiroshima and Nagasaki, the victims of A-bomb explosions. I am proud to count Jack Schull among my very dear friends with whom, I still have the pleasure of discussing a wide array of scientific problems and learning a lot from his very rich experience in the field. I am both honored and thrilled to see him here with us today.

After these personal reminiscences, I now wish to change gears and move on to the substance of my presentation today, namely, emerging perspectives in radiation genetic risk estimation. I will use the word "genetic" to mean "hereditary" from now on since genetic is less cumbersome than hereditary. There is no need to belabor the fact that spontaneously-occurring gene mutations and chromosomal aberrations, if they occur in the germ cells of individuals, can cause genetic diseases in the progeny or the fact that ionizing radiation has been found to induce similar types of changes in all organisms adequately investigated in this regard. Putting these two facts together, early on, it was concluded that exposure to radiation can cause an increase in the frequency of genetic diseases in the population. This concept has been

and still remains at the core of all our efforts. The questions that follow are: how much increase? Is it trivial, small or substantial? Over how long a period of time? To be sure, these questions are not new, but they assumed an unprecedented seriousness in the aftermath of World War II when nuclear weapons were deployed over Hiroshima and Nagasaki.

Geneticists have been tackling these questions for nearly 50 years and have been able to provide only provisional answers. The reasons for this state of affairs are not hard to seek: (i) our goal is to estimate the risk of radiation-induced genetic diseases in humans; (ii) we still need to rely on mouse radiation data for this purpose, but these data are on radiation-induced mutations, but not on genetic diseases; extrapolation from mouse to man involves a number of assumptions and associated uncertainties; (iii) in the human studies on the children of survivors of A-bomb explosions in Hiroshima and Nagasaki, no statistically demonstrable adverse effects of parental radiation exposures have been found and (iv) laboratory populations of mice, rats and swine exposed to radiation generation after generation have shown no progressive deterioration of health and well-being and no accumulation of harmful mutations with time.

The scientific landscape is now rapidly changing. We are now in a much better position than say a few years ago, to integrate the findings from human and animal studies and explain why in spite of the compelling evidence for the induction of mutations by radiation in all biological systems, we have not seen any radiation-induced genetic disease in humans either in the Japanese or other human studies and why the animal population studies have been negative. We are now in a period of transition from the purely classical approaches used thus far in risk estimation to those which will increasingly include the insights emerging from a field pivotal to radiation genetic risk estimation, namely, human molecular biology; the latter have already enabled us to restructure the conceptual framework and reformulate the critical questions in this field. In order to appreciate as to how this is so, let us first take a look at what genetic risk estimation is about and what we have achieved using the classical approaches. The general equation

used for risk estimation is a good place to start:

Risk per unit dose of radiation = P x [1/DD] x MC

In this equation, P represents the baseline frequency of genetic diseases of interest, 1/DD = relative mutation risk per unit dose and MC = the responsiveness of the disease class to an increase in mutation rate and is called the mutation component. Let us now examine each one of these and see where we are.

The quantity P is the baseline frequency of genetic diseases. These diseases are of three different kinds, namely, mendelian, chromosomal and multifactorial. Mendelian diseases are those due to mutations in single genes and show predictable patterns of inheritance; these are called mendelian diseases after Mendel who discovered the laws of heredity. Examples include, myotonic dystrophy, polycystic kidney disease, cystic fibrosis, hemophilia and Duchenne muscular dystrophy. Others such as Cri du chat syndrome, Down syndrome, Edwards syndrome etc are due to structural or numerical abnormalities of chromosomes. Multifactorial diseases, as the name implies are due to a complex interplay of multiple genes and environmental factors. Examples include the common congenital abnormalities which are present at birth such as cleft lip and cleft palate, neural tube defects and chronic diseases of adults such as diabetes, coronary heart disease, essential hypertension, allergy, asthma and many others you will be familiar with. These "run" in families but do not show mendelian patterns of inheritance i.e., the risk to first-degree relatives is much less than what is known for mendelian diseases.

Current estimates indicate that about 2.4% of children born alive are afflicted by one or another mendelian disease, about 0.4% with detectable chromosomal diseases, and at least 71% with multifactorial diseases (i.e., 6% congenital abnormalities and 65% common chronic diseases). Note that the multifactorial diseases constitute the predominant load of genetic diseases which human populations carry.

The second quantity in the risk equation, 1/DD, is the relative mutation risk per unit dose. The denominator of this fraction, DD, is

the abbreviation for what is called the doubling dose i.e., the dose of radiation that is required to produce as many mutations as those which arise spontaneously in a generation. It can be determined experimentally and is obtained by dividing the average spontaneous mutation rate in a set of genes by the average rate of induction of mutations in these genes. Mouse data on coat color mutations have been used for this purpose.

The DD which has been used until now is 100 rads (= 1 Gy) for radiation types such as X-rays and gamma rays delivered chronically or at low doses, the radiation conditions generally used for risk estimation. Note that since 1/DD is a fraction, a small DD means a high relative mutation risk and a large DD means a small relative mutation risk per unit dose.

When I say that the DD is 100 rads, I do not imply that we wish to make risk estimates at 100 rads which is a pretty large dose. Our interest in risk estimation at the population level is at low doses such as one or a few rads of radiation i.e., of the magnitude one may receive from diagnostic radiology or from other peaceful uses of nuclear energy.

The third quantity in the risk equation is MC or the mutation component. It is a measure of how responsive a given class of diseases will be to an increase in mutation rate due to radiation. Because of differences in the relationship between mutation and disease, the actual MCs as well as the procedures used to estimate them are different for mendelian and multifactorial diseases. In the case of mendelian diseases, this relationship is straightforward. Consequently their MC can be easily estimated on the basis of existing population genetic theory. For multifactorial diseases, howevcr, the relationship between mutation and disease is complex, and in the absence of theory or models of how they are maintained in the population, which was the case until recently, MC could not be estimated and therefore risk prediction was difficult.

This situation has now changed. Within the framework of an ICRP Task Group, we have been able to develop genetic theory and a mathematical model to estimate MC for multifactorial diseases. This

represents an important step forward in risk estimation for these diseases which has remained a neglected territory all these 40 years. Our results show that MC for these diseases is very very small, of the order of 0.01 to 0.02 in the first generation following radiation exposure. This means that even if the other two quantities, namely P and 1/DD remain the same as those used in the earlier years, the risk of multifactorial diseases will be very small at low doses of radiation exposure, compared to their natural prevalence. Two weeks ago, the report of my Task Group was approved by the main Commission of ICRP in Stockholm and is scheduled for publication by Elsevier as an official document; this will set the stage for revisions of genetic risk estimates in UNSCEAR and for the new recommendations planned to be issued by ICRP within the next 4 to 5 years.

I now move on to consider how molecular biology has impacted on the field of genetic risk estimation. Again, as before, a good place to start is the risk equation:

$$\text{Risk} = P \times [1/DD] \times MC$$

As you would recall, P stands for the frequency of the disease class we are interested in. The diseases include in P are those that are societally relevant. The assumption implicit in multiplying P, by the other two quantities is that these diseases will increase in frequency as a result of radiation-induced mutations. Otherwise, it makes no sense in doing that. Obviously, this can only happen if spontaneous and radiation induced changes and the mechanisms of their origin and induction are similar. Neither of these appears to be the case.

Naturally-occuring disease causing mutations include small changes in the genetic material, the DNA, called point mutations, and small and large deletions, often limited to the gene. Their mechanisms of origin are dependent on the DNA organization of the gene in question and vary between genes. The sites in the gene at which these occur are non-random and there are a number of specificites.

In contrast, although the types of genetic changes induced by radiation in experimental systems are broadly similar to those which

underlie naturally-occurring diseases, the radiation spectrum is dominated by multigene deletions. Since radiation produces mutations by random deposition of energy, one can assume that the probability of inducing a deletion is the same for all regions of the genome; however, the probability of recovering an induced deletion in a viable but affected livebirth seem more dependent on what gene functions have been lost and whether such loss is compatible with viability. Further, one does not expect that radiation will produce precisely the same specific types of changes in specific genes which nature has perfected over millenia. So, the rate of recoverable deletions which are compatible with viability of livebirths must be much lower than the rate at which they are originally induced. This is probably the principal, if not the only reason why induced genetic diseases have not been observed in humans.

If this were true, how come we have been enormously successful in radiation mutagenesis studies with a multiplicity of experimental systems? In retrospect, it is clear that majority of genes at which induced mutations have been studied in these systems are non-essential for survival and also happen to be located in genomic regions which are non-essential for survival, so that induced mutations could be recovered and studied, an incredibly fortunate coincidence indeed! Not unexpectedly, studies in which specific point mutations were looked for or which involved essential genes such as the histocompatibility genes have been negative.

You can readily see the implications of these results for the doubling dose estimate. Since the DD is a ratio of spontaneous and induction rates of mutations, if the numerator, namely, the spontaneous rate stays the same and the denominator, namely the rate of recoverable mutations in the human disease-causing genes is lower than that of the mouse genes studied, the DD will become *higher*. This means 1/DD will be lower than 1/100. How much lower cannot be said as yet without further analysis. The real problem is some what more complex.

Do all these mean that there are no genetic risks of radiation? Have we been chasing a phantom all these years? What one can say now is that the risk of radiation-inducible genetic diseases as perceived

through the "prism" of naturally-occuring ones is probably much smaller than hitherto assumed. Obviously, with around 80,000 genes in the human genome, there ought to be genes in our genome which have the attributes of genes studied in experimental systems in terms of their response to induced mutations, except that we have not found them yet. The familiar dictum applies here: if you know what you are looking for, you may or may not find it. But if you don't know what you are looking for, you may never find it. It is not a phantom chase since looking for genetic diseases of societal importance and not finding them provides a sense of reassurance in the minds of geneticists and public alike.

But there is certainly another side to the whole question of genetic risks or what I will call alternate reality. As I mentioned earlier, radiation produces genetic damage in the genome by random deposition of energy. This is another way of saying that radiation does not have the wisdom to know that genetic risk estimators are interested in diseases of societal importance nor is likely to respect that we have classified these diseases into mendelian, chromosomal and multifactorial for our convenience. It will produce genetic damage any way. Some of this damage may be incompatible with viability and will be eliminated as early embryonic losses or abortions and hence lost to view. Some of this damage, however, may be compatible with viability and therefore is potentially recoverable in the offspring if one knows what to look for, bearing in mind that the recoverable damage may be induced in regions of the genome for which we have, as yet, no "windows". How are they likely to manifest themselves? In genetic parlance, what are their potential phenotypes?

Some insights into this question come, again, from human molecular biology, namely studies of naturally-occurring human microdeletions. Most, although not all, of these deletions encompass multiple genes and in molecular terms, can be several megabases of DNA. They have been identified because of their clinical phenotypes in livebirths. Well over 30 of these are known in practically all human chromosomes, but their distribution is non-random which is to be expected considering that gene density is not uniform in all chromoso-

mes. The important point however is that despite their occurrence in different chromosomes they share some common attributes. These are multisystem developmental abnormalities, mental retardation, usually growth retardation and dysmorphic facial features. Since embryonic development and brain function are two human processes which depend on the greatest number of genes — which is why fetal brain cDNA libraries are often used for screening at the molecular level — it seems plausible that these functions should be the ones most vunerable to loss of function of several genes.

Prompted by these findings with human microdeletions, Bruce Cattanach and colleagues in England undertook extensive cytogenetic analyses of growth retarded mice ("runts") recovered in their mutation experiments. It was found that between 10 to 15% of these mice had large multigene deletions or other abnormalities and in different chromosomes; they were non-randomly distributed. The principal inferences from human and animal studies, therefore, are that (i) growth and developmental abnormalities are likely to be among the main manifestations of damage to the genome as a whole although not formally classified as "genetic diseases"; these are probably more important than mutations in single genes which the DD method aims to quantify and (iii) the phenotypes of genomic damage are not necessarily as "clean" as those of mutations in single genes. Although these arguments seem compelling, I need to critically examine the available empirical data in animals and humans in the light of genome organization and function to reach firm conclusions. This work is underway.

To summarize the principal scientific points of this lecture: We now have a model and method, for the first time in 40 years, to make risk estimation for multifactorial diseases; the estimated risks appear to be quite low. I argued that the radiation risk of mendelian diseases is probably smaller than assumed until now. I have presented a case for taking into account the consequences of genomic damage. Most importantly, I tried to illustrate how advances in human molecular biology are reshaping our ideas about genetic risks of radiation exposure and my firm belief that progress in genetic risk estimation in the coming decades is going to be intimately linked to and spearheaded by advan-

ces in human molecular biology and the development of model systems such as the mouse which will enable us to delineate gene functions, or as the molecular biologists call this endeavor, "functional genomics". You were exposed to some of the exciting aspects of this field by Rick Woychik earlier in this symposium.

I did not present any numerical estimates of risk because the task of integrating all the available information into a coherent mutually consistent risk estimate remains. I am currently working on these. The international organizations I mentioned earlier and many scientific colleagues around the world are very interested in this theme and very supportive of what I am engaged in so that together we can set the stage for further progress in the next decade. This endeavour is undoubtedly challenging, but also sometimes frustrating because of what I call the tyranny of insufficient empirical data.

Soon after his discovery of the mutagenic effects of ionizing radiation in Drosophila in the late 1920s, for which he received the Nobel prize for medicine in 1946, Muller started to alert the medical profession to the genetic consequences of carelessly and avoidably exposing the human gonads to radiation. Muller was not only one of the greatest intellects of this century, but also a great humanist; he was genuinely concerned about human welfare. The field of radiation genetics prospered. From about the mid-1950s onwards, the genetic effects of radiation effects became an integral component in radiological protection recommendations by ICRP and other organizations. Now, towards the end of this century, the advances in our science suggest that genetic risks of radiation exposure at low doses are probably not at as high as Muller feared they might be. I believe that this is an importantant achievement of 20th century science.

Ladies and gentlemen, having now come to the end of my lecture it is my pleasant task to thank the authorites of Leiden University, the Leiden University Medical Centre, Dr. Vermeer, our Dean, the J. A. Cohen Interuniversity Institute for Radiopathology and Radiation Protection, and the members of the department of radiation genetics and chemical mutagenesis for their support and understanding over these years. Earlier, I mentioned the names of some of my teachers

and colleagues who have had significant impact on my scientific career. Now I would like to thank Andrew Czeizel from Budapest who helped me to broaden my interest and knowledge about human congenital malformations and Ranajit Chakraborty at Houston with whom I have been, for the past nearly 10 years, re-exploring population genetics which was my first passion when I started doing science in the 1960s. Ranajit is a brilliant population geneticist and a great friend. Together, we have been able to build mathematical models for estimating the risk of multifactorial diseases which I mentioned in my lecture and also for assessing the impact of genetic predisposition to cancer on radiation cancer risks, one of the current problems in radiation carcinogenesis which I did not have time to go into in this lecture. I thank him most heartily for all that I was able to learn from him.

I am deeply grateful to Natarajan, my colleague here, for personally helping me to admire the magical kingdom of mammalian cytogenetics several years ago, and Paul van Buul who helped me to carry out several joint cytogenetic studies. The common denominators in my Drosophila and mammalian cytogenetic work are Annemarie van Duyn and Marjan Loos whose diligence and care in the conduct of experiments were responsible for what ever I was able to do in these fields. Jane Pleging is, and Joost van Urk was until she retired recently, a great help at the secretariat, especially when I came up with enormous manuscripts and tables for typing which they cheerfully did. Anton de Groot and Mathieu Niericker have helped me immensely at critical junctures when I panicked in ignorance when my computer did not do what I wanted it to do and Theo Wand, our photographer, who has made good quality slides for me for over 30 years for my lectures all over the world, including the ones you are seeing today. He is a loyal friend. To all of them, I am very grateful.

The pursuit of science always costs money and over more than 2 decades, the commission of the European communities, now the European union, has generously supported my scientific research; for a period of three years, I had also the good fortune of receiving a grant from the Atomic Energy Control Board of Canada in Ottawa.

I wish to thank Paul Lohman, Wouter Ferro and Marijke

Steenbergen, Albert Pastink, Bert van Zeeland and Margaret Zdzienicka of our lab and Wim Passchier from the Health Research Council of The Netherlands, for planning and organizing this occasion, the MGC, Gezondheidsraad, IRS, Leiden University and Elsevier which lent financial support to make this possible; I am very much indebted to all the speakers and the chairmen and the members of the audience who took time off from their busy schedules to be here today. Finally, human genomes as all others, need a proper environment to function effectively, starting with home. I would certainly not have been able to pursue my dreams in science without the unfailing support and the environment created by Kokila, my wife of 41 years and our 4 children. In fact, I feel blessed in this respect. Despite our Indian genomes and the associated phenotypes, we do feel "Dutch", a tribute to the great qualities of assimilation in this country. I do share with my family the overall concept "it ain't much if it ain't Dutch" although I would not want to discuss it elsewhere. Thank you all very much for your patience.