RADIO-INDUCED GENETIC RISK ESTIMATED

No hereditary effect of ionizing radiation has yet been observed in the progeny of irradiated persons. The evidence suggests that there is no increase in pathologies which can appear at first generation dominant. Researchers now have to determine to what extent this is also the case for transmitted pathologies, which would appear only after a certain number of generations recessive. Research on these effects, the incidence of which may only be extremely low, is amply justified if only to determine whether certain individuals are more predisposed than others to particular pathologies.

Several generations of a Japanese family. The exact impact of recessive mutations is assumed to be nearly nil but it can only be properly assessed from the fifth generation after the causal event.

K. Straiton/EXPLORER

After the nuclear explosions of Hiroshima and Nagasaki possible hereditary effects of the exposure to **ionizing radiation** were dreaded, yet they elicit only moderate interest among the scientific community today. The main reason for this is simple: neither animal studies nor **epidemiological** surveys on irradiated human populations have shown any significant increase in hereditary pathologies at received **doses** compatible with survival and procreation. Beyond a certain dose level, gonads and **germ** cells are disabled and can no longer function.

Insofar as direct carcinogenic effects were detected in the same conditions,

scientists understandably concentrated on the study of radio-induced malignant cell transformation (see *Radio-induced cancers*).

However, it seems it is now time to come back to the question of long-term effects, since under the conditions in which earlier work was carried out it may not have been possible to detect delayed effects. Recent advances in human genetics, in particular, now call for the reappraisal of certain concepts that were formerly well-accepted, but which may have biased early approaches.

Basis of current estimates

The estimates that can be made today concerning the hereditary effects of ionizing radiation are based first on data from animal studies, and second on epidemiological findings.

Data from animal studies

The animal research data used by the main international bodies, e.g., the International Commission on Radiological Protection (ICRP), and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) come essentially from two laboratories, one at Oak Ridge, USA, and the other at Munich, Germany, where very important experiments were carried out in the fifties, sixties and seventies.

In the absence of a molecular approach, which was not possible at that time, work focused on the straightforward detection of abnormal **phenotypes**. Of course this approach ignored at the outset all psychic impairment or effects linked directly to aging, whereas it is today acknowledged that such alterations often accompany hereditary illnesses.

In the Oak Ridge laboratories the research groups headed by William L. Russell decided to study radio-induced skeletal malformations in mice, primarily because of the ease with which they could be detected at birth. At that time scientists thought that almost all hereditary infections were congenital(1). A second reason for this choice was the idea that these malformations had a **dominant** genetic origin, i.e., that they resulted from the alteration of a single **allele**, which meant the phenotype would appear in the first generation. However, these two assumptions were partly wrong: it is now proven that a large proportion of hereditary illnesses are not congenital. Many observed congenital skeletal alterations have been found to be due to chromosomal aberrations, rather than dominant point **mutations**.

A second series of important experiments was conducted at Munich by Ehling's group, who focused on an eye disorder, cataract. The choice of this phenotype was based on the same principles: congenital character, dominant trait and easy detection.

Many other studies have of course been conducted. They cannot all be cited here, interesting though they are. All these results were pooled by the various international commissions, which after many extrapolations, produced risk level estimates. As one example of these, in its 1977 report UNSCEAR estimated the dose required to double the number of mutations at 1 **gray** (Gy). Twenty years later the same body concluded that in the first generation after irradiation of parents with 0.01 Gy, an excess of some 50 illnesses with a hereditary component could be expected per million persons, i.e., an incidence of 0.5% for 1 Gy exposure.

Epidemiological data

Two sorts of epidemiological data exist; the first one concerns the progeny of individuals irradiated either medically or accidentally for a short time - fortunately only a small number of cases - or deliberately in the two nuclear bomb attacks on Hiroshima and Nagasaki (Japan). Hence these studies have mainly concerned the populations living around these cities. The conclusions are simple: no detectable increase in the incidence of hereditary pathologies.

(1) That appears at birth.

Other data derives from studies on populations living in regions with «strong» background **radiation levels**. We may add in passing that these studies attracted strong media attention whenever an increased incidence of pathology was reported, but no attention at all when no such increase was found. In fact, it must be acknowledged that the results are conflicting and so unreliable, since there are no adequate records nor sufficient medical monitoring in those areas with high natural radiation levels that have been studied.

The data from Hiroshima and Nagasaki thus forms the sole basis on which we can attempt to define a threshold level below which no hereditary effect is observed, which is unhelpful.

Why revise these estimates?

Although still theoretical, a number of related considerations have prompted the re-evaluation of the current estimates in the light of advances in fundamental and human genetics. Progress in human genetics is advancing almost exponentially at present, as shown in Figure 1, which gives the number of **genes** identified and located on human **chromosomes** between 1966 and 1997. A similar pattern is seen for the mouse, which is the «model» mammal for genetics. Given that the large-scale experiments on long-term effects date back to before the eighties, it is clear that the lack of information available at the time could have introduced marked bias. The possibility of such bias is indeed evident from Figure 1: the number of genes assigned to the X chromosome was multiplied by only four between 1960 and 1997, while the number assigned to **autosomes** went from 0 to about 4,000 in the same time! The major experiments on which the evaluation of genetic risk relies were thus conducted at a time when the genetics of mammals was in its infancy, suggesting that many of the early conclusions now need reviewing.

The progress made in fundamental genetics has been just as important. The mechanisms involved in the heredity of mammals began to become known only with the development of molecular bio-

logy, in particular from the eighties. How a particular **DNA** alteration will influence a particular phenotype deserves to be re-examined in the light of the most recent findings. The simple notion of **dominance** (one affected allele, one effect), and **recessiveness** (two affected alleles, one effect) has considerably evolved over the years, because it depends on how powerful are the methods of analysis that can be implemented.

This initially very formal notion lost much of its importance in the sixties and seventies with progress in biochemistry, which made it possible to detect the effects of gene dosage, at intermediate levels between strict dominance and recessivity.

Progress in molecular **oncology** has given new impetus to this notion. A character trait such as hereditary predisposition to cancer may appear dominant because it manifests itself in **heterozygous** individuals, i.e., those bearing a single altered allele, whereas it is recessive at the cell level, because the second allele has mutated or been lost in the cancer cells. This two-step process, one of which takes place as the individual ages, illustrates how restrictive was the assumption that hereditary disorders were congenital.

Induced alterations to DNA and their consequences also deserve careful consideration. Five types of alteration can be listed. First, non-**transcribed** lesions, which result in simple DNA polymorphism. Second, transcribed lesions, which cause non-pathological variations in phenotype. Third, lesions that suppress the function of the affected allele, with no effect if the other allele compensates for it (recessive effect), or which display a «gene dosage» effect (dominant effect). Fourth, lesions that change the message in such a way that the product of the mutant allele competes with the remaining normal allele (negative dominant effect). Fifth and last, lesions can extend to several genes, **deleted** or duplicated, thus forming alterations at the chromosomal scale (dominant effect).

For obvious reasons dominant mutations are easily recognized because they are identified in the first generation by the effects they produce, unlike recessive mutations. This results in considerable distortion of our knowledge of DNA alterations. Thus in the catalog of Dr Victor A. Mc Kusick of John Hopkins University (1998), USA, there are three times more dominant autosomal traits identified in man than recessive ones, but the ratio is inverse for traits linked to the X chromosome. The reason for this is that recessive mutations are not easily discerned unless they are carried by the X chromosome, because then they are seen in males, who possess a single X chromosome (sex-linked pathology). Thousands of recessive autosomal diseases may thus be still unidentified. This difficulty can play a very important role in the evaluation of genetic risk due to radiation, as we will see further on.

There is another unknown that is at least as important. After exposure to radiation, like any other **mutagen**, the ratio of lesions with recessive effects to those with dominant effects is not yet known. Since the loss of the function of an allele (recessive mutation) does not need a high specificity (causing a failure in an integrated system is easy, whereas modifying a function (dominant mutation) requires more narrowly targeted damage), the vast majority of mutations might be expected to be recessive. Some experimental evidence points this way, but results are unfortunately biased by the approaches used. It is also established that, in cancer cells, the observed mutations are very often recessive, causing loss of function. Lastly it must be borne in mind that a recessive mutation in a single copy remains neu-

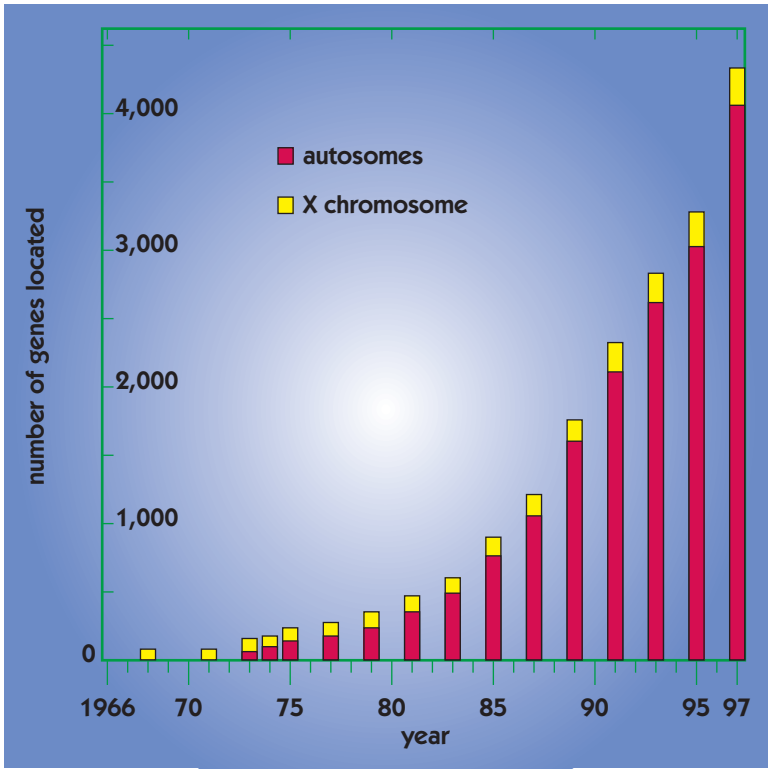
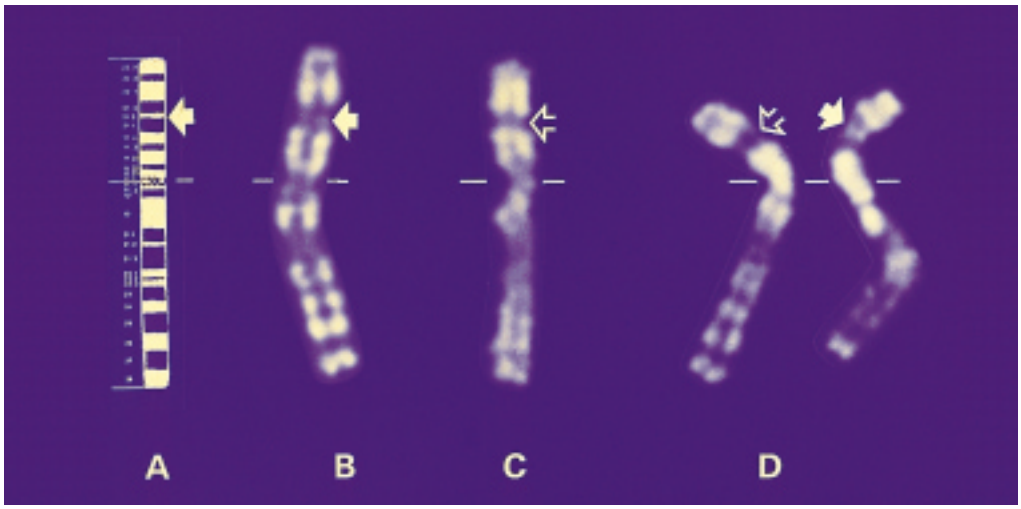


Figure 1. Time course of the numbers of genes located on human chromosomes in the last thirty years (from Victor A. Mc Kusick, Mendelian Inheritance in Man, 12th edition).

Location on chromosome X of the gene DMD responsible for Duchenne's myopathy. A: diagram of a normal X chromosome. B: normal X chromosome. C: X chromosome with deletion affecting gene DMD in a boy with the illness. D: X chromosomes of healthy mother carrier, one of which exhibits a deletion.



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tral. It will therefore not be weeded out, making it easily transmissible.

From the fifth generation?

So how valid are the studies on the descendants of the survivors of Hiroshima and Nagasaki if, as might reasonably be supposed, the induced mutations are mostly recessive? The family tree in Figure 2 shows that no conclusion can yet be drawn as regards recessive mutations. No effect would be expected in the first generation (1946 to 1966). However, heterozygotes carrying various mutations, all of them different, because any of about 50,000 genes may be affected, must exist. Each of these would have a 50% chance of transmitting that mutation to each offspring. The same mutation would thus be liable to be transmitted in siblings (second generation, 1966 to 1986).

As brother-sister unions may be assumed to be exceedingly rare, the third generation (1986-2006) has no chance of including **homozygous** carriers of the mutation. Union between first cousins, which is not recommended, is very infrequent, so the number of homozygotes will be very small in the fourth generation (2006-2026). Thereafter the incest taboo will not prevent unions between more distant cousins, and so it will be in the fifth generation (2026-2046) that a detectable outcome might be expected. Hence it is much to soon to assert that there is no effect on hereditary pathologies in these populations. All that can be said with certainty is that there has been no increase in pathologies with dominant transmission.

Current research on mutations at the molecular level is also important. Ongoing studies are discreetly challenging earlier evaluations. It is unlikely that high levels of genetic risk will be predicted, but it would be irresponsible simply to ignore the risk, however small. Fortunately for the descendants, the sterilization of the gonads, an immediate consequence of heavy irradiation, limits the genetic risk. However, any chronic increase can have consequences, even though they may not show up in epidemiological surveys, because these are not powerful enough to detect rare events in large populations, especially if they are not medically controlled. Furthermore they have no predictive value.

The resources of molecular biology are rapidly progressing. Detecting a mutation has become commonplace in a defined genetic context. This will become just as commonplace at the scale of the whole genome once the global analysis systems (DNA chips) are fully operational (box). Hence it was logical that a department of functional genome analysis and a laboratory for the study of the radiosensitivity of germ cells should be developed at the French Atomic Energy Commission (CEA) in the field of radiobiology research. At a time when public opinion is concerned about the management of nuclear waste and the possible effects of slight increases in chronic exposure to ionizing radiation, it is important not to be taken unawares by an unforeseen increase in the incidence of recessive mutations.

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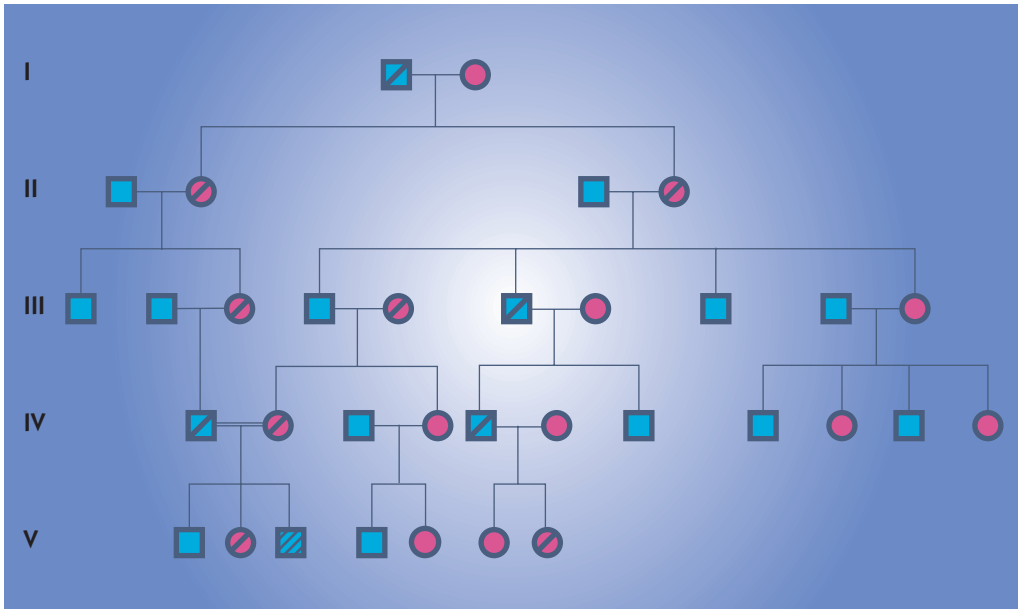

Figure 2. Theoretical family tree showing the transmission of a recessive mutation acquired in the gonads of a parent of the man in generation I. Square: man. Circle: woman. Slash: healthy carrier of the mutation. Hatching: affected homozygote. Double horizontal line: consanguineous union of second cousins.

The prowess of DNA chips

The most recent tool for the overall analysis of genetic expression is the DNA chip. It is able to identify hundreds, soon thousands or tens of thousands of DNA samples in a single operation.

On a treated glass or silicon support, comparable to those used in the manufacture of electronic microprocessors, are deposited fragments of DNA (succession of **bases A**, **T**, **G** and **C**) specific to the genome of a species (synthetic **oligonucleotides** or DNA sequences amplified using the polymerase chain reaction (PCR) technique). These «probe» strands constitute hybridization sites that will be recognized, according to the principle of the double helix (box A, ***DNA molecule, heredity vector***), by «target» cell DNA (succession of complementary bases T, A, C and G), if they are present in the sample studied. To analyze a biological sample, it is necessary to prepare targets, label them with a fluorescent molecule and then leave them to «hybridize» on the DNA chip. Thus by locating and then quantifying the fluorescent signal on each DNA probe deposited on the chip, the state of the cells is characterized in one step in the test sample, the extent of the analysis being dependent on the number of probes deposited on the chip.

In radiobiology in the field of irradiation impact assessment *global analysis methods* using such chips will make it possible to reveal radio-induced genetic mutations. They will also serve to study cell response to stress, characterize the early effects of radiation, discern radiosensitivity markers and improve the understanding of dose-effect relations.

The Biochips program of CEA now aims to develop chips comprising up to 100,000 hybridization sites, against 128 in the first biochips designed with Cis bio International. Different Divisions of CEA are participating, essentially the Life Sciences Division and the Advanced Technologies Division through the Electronics, Technology and Instrumentation Laboratory (Leti).

In 1998 CEA extended its industrial partnerships, e.g., by forming an association with bioMérieux, a company specialized in medical diagnostic products.

High-density biochip, developed as part of the Micam program, carrying 8,100 hybridization sites.

CEA/Studio Pons