Revisiting next generation effects of ionizing radiation

John D Mathews[1]

Melbourne School of Population and Global Health
University of Melbourne, Australia
mathewsj@unimelb.edu.au

Summary

It is almost 100 years since HJ Muller first showed that X-rays caused germline gene mutations in fruit flies. In the generations since, mutations have been studied in most species, but there is continuing uncertainty about the importance of ionizing radiation as a cause of harmful germline mutations in humans. It is important to try and measure the magnitude of any such next generation risks, not least because medical radiation contributes substantially to individual radiation doses in many countries. We already know that ionizing radiation can cause *de novo* gene deletions and duplications, collectively known as copy number variants (CNV). More recently it has been shown that *de novo* CNV are predictive of adverse outcomes such as intellectual disability, autism, congenital anomalies, premature mortality and cancer. Thus, we have a plausible causal pathway that starts with low dose radiation, prior to conception or post-zygotically, and ends with a *de novo* CNV mediating adverse effects in any offspring that carries it. If this pathway is important, there should be a correlation between the timing of radiation doses to parents and the risk of genetically mediated adverse effects in their offspring. To test this potentially important pathway, we have planned a national record linkage study to estimate parental doses of medical radiation and to assess whether doses before conception or during pregnancy are predictive of adverse outcomes amongst some seven million Australian offspring.

Introduction

The possibility that ionizing radiation could cause germ-line mutations and genetic disease in human populations was first considered seriously in the years after the atomic bombings of Hiroshima and Nagasaki, and later during the cold war era of atmospheric testing of nuclear weapons[1, 2]. Public concerns were re-awakened following nuclear accidents at Chernobyl and Fukushima[3]. Despite such long-standing community concerns, most populations today are much more exposed to radiation from medical procedures than from nuclear fallout or reactor accidents[4]. Unfortunately, we still do not know whether germline mutations attributable to low dose radiation have had adverse effects in exposed human populations.

What do we know about mutation?

The most frequent *de novo* mutations (DNM) are single nucleotide variants (SNV)[5-7]. These point mutations can be caused by replication errors and by oxidative (free radical) damage to DNA. SNV arise at a rate of about 44-72 mutations per generation, mostly in the paternal germline. SNV increase with parental age, most strikingly for fathers. Although most SNV appear to be selectively neutral, a small proportion cause genetic disease.

In contrast, gene deletions and duplications, known as copy number variants (CNV) are much less frequent than SNV[5]. CNV arise at an average rate of 0.01-0.02 per generation. However, because CNV are large, with sizes ranging from 50 bp to 10 Mbp or more, the probability that a new mutation will have adverse genetic effects is much greater for CNV than for SNV mutations. As a result, CNV contribute much more genetic variation than SNV. Most CNV behave as functional dominants. The larger the size of a *de novo* CNV, the greater the probability that it will be lost through purifying selection by way of

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pregnancy loss, still birth, congenital defects, genetic disabilities or premature death[5]. Indeed, the most harmful CNV mutations do not propagate beyond the first generation, so that their phenotypes cannot show familial aggregation. In earlier times, without genomic sequencing, those phenotypes were not necessarily regarded as genetic in origin. (See also Box 1 below.)

Children of parents exposed at Chernobyl

There is little direct evidence of low dose parental radiation affecting germ-line mutation or adverse outcomes in humans[1, 3]. In their important paper, Yeager and colleagues[3] used whole genome sequencing (WGS) to identify *de novo* mutations (DNM) in children of parents exposed to Chernobyl radiation before conception. The DNMs detected were predominantly single nucleotide variants (SNV), which did not increase in frequency with estimated parental radiation doses. The negative results in the Yeager study could have been due to lack of statistical power, as there were only 130 family trios studied. Furthermore, because of the time delay between Chernobyl and specimen collection, any rare but harmful mutations could have been lost through premature death.

Although the Yeager results were consistent with earlier studies in humans[8, 9] and mice[10] in showing no increase in *de novo* SNV following parental radiation exposure, those earlier studies did show an effect of parental radiation on CNV incidence.

Looking ahead

On balance we suggest that if parental radiation does have genetic effects on the next generation, these are less likely to be mediated by SNV and more likely to be mediated through copy number variants (CNV), which are characteristic signatures of ionizing radiation[1, 8, 10].

Unfortunately, the Yeager study[3] was not designed to report on CNV, so that a definitive test of the CNV hypothesis will require a larger study using array technologies to measure CNV mutations more efficiently. Such a study would be of most salience for public health if it were able to assess any next generation mutational risks attributable to parental X-rays and scans.

An epidemiological approach can bypass the genetics

We have previously used deidentified health records to estimate organ doses from medical irradiation[11], and used national record linkage to identify increased cancer risks attributable to CT scan exposures in childhood and adolescence[12, 13]. This experience has encouraged us to plan a national epidemiological study to link parental records of medical radiation to the health outcomes of their children. This will allow a direct test of the hypothesis that parental radiation doses, before conception or during pregnancy, predict adverse outcomes in their offspring. Estimates of the magnitude of any next generation burden will allow us to assess the scope for prevention, for example by reducing medical radiation exposures for persons who may yet still reproduce.

Designing a national linkage study in Australia

Strategy: Electronic birth records can be accessed for up to 7 million children born between 1985 and 2020, allowing records of children to be linked to parental records. Parental health records can be used to estimate doses of radiation from medical procedures and their timing in relation to conception and pregnancy dates. (See Box 3 below). Child outcomes can be assessed by linking to records from the National Death Index, the Australian Cancer Database, Medicare, and to perinatal, disability and other specialist registers. (See Box 2 below).

Which adverse outcomes to include? In our epidemiological study we will not have access to population-wide molecular genetic diagnoses. Accordingly, we will define broad phenotypic outcomes that are arguably genetic in origin: perinatal morbidity, intellectual disability, autism, congenital anomalies, premature mortality or cancer.

Dose responses, target sizes and statistical power: Radiation-induced mutation rates for germline genes are poorly understood in humans. Authorities have based human estimates on results from irradiated male mice. The average mutation rate, 1.09×10^{-5} per functional locus per Gy, leads to an assumed 'doubling dose' of about 1 Gy[1]. That is, for an average specific locus, we would expect 1 Gy of radiation to double the rate of mutation. For a typical dose of 10 mGy, and assuming linearity, we would expect only a 1% increase in mutation rate, which would be undetectable.

However, we need to remember that a deleterious outcome might be caused by genetic damage anywhere in the genome, with many target loci at potential risk. For families, the aggregate phenotypic risk from parental radiation is what really matters. The essential question is: "What is the overall (deleterious) effect of a given dose of radiation if we consider the whole genome to be the target?" By pooling outcomes, we are addressing that basic question, and the increased numbers of outcomes will provide increased power to reject the null hypothesis of "no effect". Of course, the pooling implicitly assumes that grouped outcomes are affected in similar ways by parental radiation doses.

What power might we have in our study? In the simplest case, let us assume that we have 5 million children of whom 2% (100,000) have at least one of the outcomes of interest. For the parents with linked radiation histories, about 10% will have had radiation in the year before conception, so that if the null hypothesis were true, we would expect about 10,000 affected children to have a history of similar parental exposure. This gives great power to detect a 5% increase in adverse outcomes attributable to parental radiation. Of course, the power will decrease as the number of specified phenotypic groupings is allowed to increase.

This scenario is also consistent with data suggesting that CNV increase after parental radiation with an excess relative risk (ERR) of 0.2-0.3 per 100 mGy[9, 10]. If multiple loci (targets) are at risk, such an ERR could easily give rise to a 5% excess of phenotypic outcomes in the offspring of exposed parents.

Box 4 (below), summarizes approaches to causal inference needed to minimize biases arising from confounding and reverse causation.

Summing up

- Low dose radiation from medical imaging could be contributing to gene mutation, to adverse health effects in the next generations, and to genetic load in future generations.
- With national record linkage we can now quantify the risks for the whole of Australia.
- If radiation-related risks are trivial, the population can be re-assured.
- If the risks are non-trivial, our study will allow the social and economic implications and the scope for prevention to be assessed. In particular:
    - The life-time costs of severe autism are some US$2.4 million per person, over and above the personal costs to families in terms of amenity and opportunities forgone.
    - Likewise, about 40% of child health care costs are attributable to genetic disorders.
    - Substantial numbers of adverse outcomes in the next generation could be prevented by progressively reducing medical radiation exposures in individuals who may yet reproduce.

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BOX 1

CNV mutations and radiation

Ionizing radiation damages DNA by causing about 30 double-stranded breaks (DSB) per genome per Gy of radiation. Most DSB are repaired, but the occasional mis-repaired lesion(s) can result in a deletion or duplication of DNA, giving a new CNV. The size of a CNV will depend upon the distance between the two DSB that are mis-joined; because of DNA coiling, this means that large CNV can form by chance, although less often than smaller CNV. This raises the possibility that CNV size will not increase in direct proportion to radiation dose. Furthermore, at larger radiation doses, damaged gametes may become non-viable, leading to infertility or early fetal loss[14], so that phenotypes seen at lower doses may disappear. Overall, larger CNV are more likely to be deleterious, leading to "purifying" negative selection and rapid loss from the population.

BOX 2

Exposure and outcome information in Australia

INFORMATION	PRIMARY SOURCE	ADDITIONAL SOURCE
Parental radiation exposures	Medicare records	State hospital records
Family relationships	Birth records	
Pregnancy loss, stillbirths and birth defects	Medicare records (eg of ultrasound tests)	National Perinatal Data Collection and birth defect registries
Child Mortality	National Death Index	
Cancer incidence	Australian Cancer Database	
Genetic outcomes	Medicare records of genetic tests and referrals (eg for autism)	Specialist genetic registers

Assessing exposures from Medicare records

- Over 4 million annual exposures to CT or NM scans, and 10 million diagnostic X-rays; 15-20% are in persons of child-bearing age.
- Preliminary estimates of gonadal doses in exposed are:
  - Testis: mean 1.2 mGy; maximum 10 mGy (shielding ambiguity).
  - Ovary: mean 8 mGy; maximum 50 mGy
  - Uterus: mean 7 mGy; maxium 45 mGy.
- Individual dose estimates depend on the organs scanned.
- Additional information will come from the timing of radiation exposures in relation to estimated dates of conception.

BOX 4

Approaches to causal inference

- Use Poisson or Cox regression to assess how organ doses predicts adverse outcomes, with adjustment for parental age, year of conception, and SES.
- Compare with outcomes from "unexposed" parents, and with unexposed "sibling" pregnancies in the same family.
- Estimate the "sensitive" period by maximizing the profile likelihood for different intervals between exposure and conception.
- Outcomes associated with prior exposures are more likely to be "caused" if there is a dose response relationship and no obvious path for confounding (eg cancer in childhood following parental exposure before conception.)
- Suspect reverse causation and confounding if outcomes or their precursors can be diagnosed by X-rays.
- Suspect indication bias if parent and child could share a (genetic) condition requiring X-rays for diagnosis or management.