# Revisiting next generation effects of ionizing radiation

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# Why should we revisit the question about next generation effects of ionizing radiation?

It is almost 100 years since Muller showed that ionizing radiation caused germline mutations in fruit flies.

*However, we still do not know whether germline mutations attributable to low dose radiation have had adverse effects in human populations.*

The possibility of adverse genetic effects in human populations was first considered seriously after the atomic bombings in Japan.

No extra mutations have been observed in the children of survivors.

Concerns reemerged with the cluster of leukemias amongst children of radiation workers in Seascale.

The Gardner hypothesis was discredited.

In many populations today, medical procedures now deliver as much radiation as comes from all other sources combined.

***If medical imaging of parents can contribute to adverse outcomes in the next generation, we should urgently re-assess risks and benefits.***

# What do we know about mutations, especially those caused by ionizing radiation?

**SINGLE NUCLEOTIDE VARIANTS (POINT MUTATIONS)**

- **FREQUENT (44-82 per generation) – 75% Paternal origin**
- **INCREASE WITH (PATERNAL) AGE**
- **SNV ARE MOSTLY NEUTRAL – RARELY CAUSE DISEASE**

**COPY NUMBER VARIANTS (DELETIONS AND DUPLICATIONS)**

- **INFREQUENT (1-2% per generation)**
- **LARGE IN SIZE (50 BP to 10 MB)**
- **MUCH MORE LIKELY TO CAUSE DISEASE, ESPECIALLY IF LARGE**
  - **PURIFYING SELECTION**
  - **INTELLECTUAL DISABILITY, CONGENITAL ABNORMALITIES, CANCER….**
- **CNV ARE KNOWN TO BE CAUSED BY IONIZING RADIATION.**

# Next generation effects of medical radiation are not fanciful because:

Genetic mechanisms to repair radiation damage to DNA are highly developed, suggesting that radiation is an important agent of natural selection.

Germline mutations, especially gene deletions and duplications, known as Copy Number Variants (CNV), contribute to outcomes such as intellectual disability, congenital anomalies, and cancer.

Low dose radiation contributes to both germline and somatic mutation. CNV are more important than SNV in causing adverse effects.

Radiation-induced mutation in humans needs to be better understood.

Radiation in pregnancy is known to increase the risk of cancer in the child.

But this risk has not been adequately re-evaluated since the introduction of CT scans in the 1980s.

# *De Novo* germline mutations (DNM) after Chernobyl

M. Yeager *et al*., *Science* 10.1126/science.abg2365 (2021).

Whole genome sequencing (WGS) of 130 children and their parents (trios) to identify de novo mutations (DNM) following parental exposure to Chernobyl radiation.

| | Mean number per child |
|---|---|
| Single Nucleotide Variants (SNV) | 72 |
| Other DNM | 18 |
| Total DNM | 90 |

No increase in mutations with parental radiation doses.

Copy number variants (CNV) were not scored.

# Why might previous studies have failed to detect next generation effects of radiation?

Deleterious effects will be rare, so that a large number of exposed individuals are needed to achieve statistical power.

Single locus studies are of very low power; better to assess grouped phenotypic effects at a given dose with the whole genome as target.

Deleterious syndromes that are of early onset may not survive to be enumerated, even in large studies, and some would not be recognized as genetic in the era before genomic sequencing.

It is possible that germline CNV mutations at low radiation doses are not seen at higher doses because of selection against damaged gametes; this would imply that dose responses are non-linear.

It is possible that any real effects of parental radiation are so small as to be negligible!

# Can the next generation question be answered?

Large numbers of people are exposed to diagnostic medical radiation – potentially at risk.

We can use medical records to estimate radiation doses for exposures before conception or in pregnancy.

We can use record linkage to assess next generation outcomes in offspring (eg intellectual disability, perinatal morbidity, cancer,  premature mortality)

We can test whether outcomes increase with parental radiation doses prior to conception or in pregnancy.

# Mutation rates, target sizes and power

The average mutation rate in male mice, $1.09 \times 10^{-5}$ per functional locus per Gy, leads to an assumed 'doubling dose' of about 1 Gy.

For a typical dose of 10 mGy, and assuming linearity, we would expect only a 1% increase in mutation rate for that locus, which would be undetectable.

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 grouping similar phenotypes together, we have more outcomes, giving increased power to reject the null hypothesis of "no effect".

Suppose we have 5 million children of whom 2% (100,000) have intellectual disability as children. 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 expect about 10,000 affected children to have a history of parental exposure.

This gives great power to detect a 5 % increase in adverse outcomes attributable to parental radiation.

# Summary

- There is continuing uncertainty about the importance of ionizing radiation as a cause of harmful germline mutations in humans.
- It is important to measure the magnitude of any such next generation risks, not least because medical radiation contributes substantially to individual radiation doses.
- We already know that ionizing radiation can cause *de novo* copy number variants (CNV) which 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 *de novo* CNV mediating adverse effects in an affected offspring.
- 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 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.

# 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 |

# Approaches to causal inference

- Separate maternal and paternal dose effects.
- 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.

# 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.

# Range of plausible dose effects

| OUTCOME | EXPOSURE | ERR/100 mGy | REFERENCE |
|---|---|---|---|
| Germline copy number variant (CNV) | Human 0.2 Gy caesium | 0.3 | Costa |
| Germline copy number variant (CNV) | Mouse – paternal 3 Gy | 0.2 | Adewoye |
| Childhood cancer | X-ray in pregnancy | 5 | Doll & Wakeford |
| Cancer | CT scan in childhood | 0.4-5 | Our study |

# De novo germline mutations – SNV and CNV

| Type of mutation | Size (bp) | Frequency (per gamete per generation) | Mutational effect of ionizing radiation | Selective effects on fitness (disease) |
|---|---|---|---|---|
| Single Nucleotide Variant (SNV) | 1 | 30 | Marginal | Very small |
| Copy number variants (CNV); deletions or duplications | Up to 10 Mb | 1-2% | Characteristic signature | Strong purifying selection (ie disease, disability and death) with large CNV |
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