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Centre for Health Effects of Radiological and Chemical Agents

UK Nuclear Test Veterans: Genetic and Cytogenetic Family Trio Study Report.

The potential effects of parental exposure to ionising radiation prior to conception—and how such exposure might influence the health of children and future generations—remain both poorly understood and scientifically contested. This issue continues to be of particular concern for ageing veterans who participated in the British nuclear testing programme more than half a century ago.

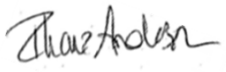
The Genetic and Cytogenetic Family Trio (GCFT) Study represents the first research initiative to collect and analyse blood samples from UK nuclear test veterans and their families. Its aim was to investigate whether any genetic or chromosomal changes in their children were associated with the fathers' historical exposure to ionising radiation. The study focused on two central questions:

- Is there chromosomal evidence of past radiation exposure in the veterans themselves?
- Do the children of these veterans exhibit differences in the number or types of DNA mutations or chromosomal abnormalities that could be associated with their fathers' presence at nuclear test sites?

The study was conducted at Brunel University of London, in collaboration with the London School of Hygiene & Tropical Medicine and the University of Leicester. It was funded by the Nuclear Community Charity Trust, supported by the Armed Forces Aged Veterans Covenant Trust.

As Principal Investigator, I am profoundly grateful to the research teams across all partner institutions whose expertise and commitment made this study possible. All contributors are recognised either as authors or acknowledged collaborators in the peer-reviewed publications included in this report. Most importantly, I extend my sincere thanks to the many families who generously participated. Their involvement has advanced our understanding of critical questions affecting both the nuclear test veteran community and the broader field of radiation effects.

This report brings together all aspects of the GCFT Study—both published and unpublished—to provide a comprehensive overview of the research, its key findings, and the recommendations that emerge from them.



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Executive Summary

This report presents the findings of the first study to investigate potential heritable genetic effects of ionising radiation exposure among UK nuclear test veterans and their descendants, using both cytogenetic (chromosomal) and genomic (whole genome sequencing) approaches. The study responds to longstanding concerns raised by nuclear test veterans and their families regarding possible health impacts linked to past radiation exposure during the British nuclear testing programme in the 1950s and 60s.

Background and Rationale

While the risks of radiation exposure during pregnancy are well documented, the possibility that exposure of the parental gametes prior to conception affecting the health of future children remains less understood and scientifically debated. British nuclear test veterans represent a unique cohort, having potentially been exposed to ionising radiation while stationed at test sites in Australia and the South Pacific. Although early epidemiological studies found no major health issues among veterans, more recent analyses up to 2017 have reported small but statistically significant increases in illness and mortality compared to a control population of veterans. No epidemiological studies have examined the health of their descendants although anecdotal surveys do report adverse outcomes.

The purpose of the Genetic and Cytogenetic Family Trio (GCFT) study was to assess for potential genetic effects of past radiation exposure. For this, we examined for chromosomal aberrations and genomic variation in nuclear test veterans and their adult children, comparing findings with controls and sub-groups within the recruited cohorts.

Study Design and Methods

Study participants: Armed services nuclear test and control veterans were selected for invitation to participate from the UK nuclear test veteran's cohort. Test veteran invitation targeted those with the highest potential for radiation exposure, including veterans involved in multiple nuclear test operations or high-risk roles (e.g. HMS Diana crew, Maralinga personnel). Control veterans were group-matched on age, service and period of service in tropical regions. In total, blood was received from 49 nuclear test and 42 control veteran family trios (veteran father, mother, and one child conceived after the father's last nuclear test deployment or tour of duty).

Techniques Used:

- *Multiplex Fluorescence In Situ Hybridisation (M-FISH)* labels chromosomes with fluorescent dyes enabling the unique identification of individual chromosomes and the microscopic detection of chromosome rearrangements. Evidence of past radiation exposure in veterans blood cells was looked for by the occurrence of simple and more complex chromosome aberrations in veterans blood cells.
- *Whole Genome Sequencing (WGS)* of DNA enables the detection of mutations in individuals. Here, WGS of veteran family trios was used to identify DNA mutations

present in a child but which are not present in either parent; termed as newly arising (de novo) germline mutations.

- *G-band and conventional karyotyping* were used to stain chromosomes from adult children blood cells to detect chromosomal abnormalities which are present in every cell from birth (termed as constitutional), and those which are present in only a fraction of cells (newly arising indicators of genomic instability).

Key Findings

Overall, the findings are reassuring. However, certain sub-groups exhibited notable findings, particularly in veteran families with higher radiation exposure risks:

Veteran Chromosomal Results:

- No significant difference in simple or complex chromosomal aberrations was seen between nuclear test and control veteran cohorts overall.
- A small subset of veterans who have a higher potential for radiation exposure (e.g. veterans at Maralinga (N=5), exhibited increased average frequencies of complex aberrations—a possible indicator of historical internal contamination with alpha-emitting radionuclides like plutonium-239.

Adult children's Chromosomal and Genomic Results:

- No evidence of any constitutional chromosomal abnormalities in children of nuclear test veterans.
- No significant difference in de novo germline mutations between children of nuclear test and control veterans.
- A statistically weak, non-robust, increase in chromatid-type aberrations (newly arising cytogenetic indicator of genomic instability) was seen in a very small group (N=4) of adult children born to nuclear test veterans with a higher potential for exposure. This requires cautious interpretation.
- The vast majority showed no significant differences in cytogenetic indicators of genomic instability between adult children born to nuclear test and control veterans.

Germline Mutation Signature Analysis:

- A small subset of families (N=8; 6 nuclear test and 2 control) showed an enrichment of a genetic mutational signature, termed SBS16, in the germline.
- This high-SBS16 subset of families showed a weak statistical association with higher average frequencies of complex chromosomal aberrations in veterans.
- A significant positive trend between the occurrence of unstable aberrations in the veteran father and chromatid-type aberrations in the adult children was stronger in the high-SBS16 subset of families. This requires cautious interpretation.

Self-Reported Health Outcomes:

- More nuclear test veteran families who were recruited to the GCFT study reported congenital anomalies in children or grandchildren compared to controls, likely reflecting participation bias.
- No statistically significant relationship was found between veteran's chromosomal aberration burden, germline mutation frequency, adult child genomic instability, and reported family health issues.

Conclusions and Implications

- For most British nuclear test veterans and their families, we found no chromosomal or genomic evidence of radiation exposure or heritable changes.
- A small group of nuclear test veterans with higher radiation exposure potential did show more complex chromosomal aberrations, consistent with internal radiation contamination as observed with other exposed populations.
- No measurable genetic effects were observed in the children of nuclear test veterans that could explain the health concerns reported.
- The observations involving mutation signature SBS16 in a small subset of families requires additional research in larger radiation exposed cohorts to investigate the biological significance, if any, of this.

Overall, the findings should provide reassurance to nuclear test veterans and their families, as we found no significant genetic impact from participation in nuclear tests.

Recommendations to advance research and support

The GCFT study contributes valuable new evidence to the field of radiation genetics and the historical record of the British nuclear test programme. While the study found no statistical evidence of heritable genetic changes, it identified areas warranting further investigation.

1. Investigate SBS16 Mutation Patterns

Despite the small sample size, the multiple observations involving families in the high-SBS16 subgroup provide a compelling and urgent rationale for targeted research funding. This should prioritise:

- investigation of mutation signatures in populations with documented radiation exposures, including from α-particle emitters.
- investigation of co-occurring environmental, genetic, or lifestyle factors that may modulate these mutation profiles.

This evidence-based approach will not only enhance our understanding of mutational processes but also inform public health strategies.

2. Implement Long-Term Health Monitoring for Descendants

To address the participation bias and high levels of community concern demonstrated during this study's recruitment, consideration should be given to targeted health monitoring programmes for descendants. Further, while this study does not provide evidence of genetic effects, further targeted research of some families may be warranted in the future.

3. Establish and expand multi-generational family trio cohorts for radiation research

To advance scientific understanding of the long-term and intergenerational effects of radiation exposure, it is essential to establish and expand structured cohorts of family trios—comprising exposed individuals, their partners, and offspring. Priority should be given to:

- Systematic recruitment of cohorts from known radiation-exposed populations, such as nuclear industry workers.
- Provision of sustained funding and infrastructure to support longitudinal data collection, biological sampling, and psychosocial assessments across generations.

This initiative will create a robust evidence base to inform future health policy, risk assessment, and public communication.

Conclusion

These recommendations aim to advance scientific understanding which will inform public health strategies and ensure support for communities affected by historical nuclear testing. As the use of medical radiation increases and global risks of nuclear exposure persist, trio-based genomic studies like the GCFT study are essential for understanding intergenerational health risks and guiding future research and policy.

Lay Summary

The Genetic and Cytogenetic Family Trio (GCFT) study was designed to address a long-standing question: could British nuclear test veterans—who participated in nuclear weapons testing during the 1950s and 60s—have passed on radiation-related genetic changes to their children?

While radiation's impact during pregnancy is well-known, less is understood about its potential long-term effects when exposure happens before conception.

The British nuclear test veterans, who were involved in testing at sites in Australia and the South Pacific, have long been concerned about the health of their families, particularly about whether their children or grandchildren might suffer from inherited health problems due to their fathers' possible exposure to radiation.

To explore this, researchers from Brunel University of London, the London School of Hygiene & Tropical Medicine, and the University of Leicester studied veteran family trios (father, mother, and one child conceived after the father's last nuclear test deployment). The study used advanced genetic techniques, including chromosome painting and whole genome sequencing (WGS), to look for evidence of:

1. Radiation-related chromosomal changes in veterans
2. Genetic alterations in their adult children

Key Findings

The GCFT study provides reassurance that, for most British nuclear test veterans and their families, there is no genetic evidence of historical radiation exposure. Similarly, for most adult children born to nuclear test veterans, we found no differences in the occurrence of chromosomal alterations.

We did see, in a small group of veterans who were potentially exposed to higher levels of radiation, some signs of complex chromosomal changes suggesting potential exposure to internalised radioactive fallout. We also identified a very small group of adult children who had indicators of unstable damage in their genome however this preliminary observation was not statistically robust, meaning this needs to be interpreted with caution.

A specific pattern of DNA changes, called SBS16, was seen in a few families (including some control families). These families partly overlapped with veterans who previously showed higher levels of complex chromosome changes, and with a small group of adult children who had signs of unstable damage. This means their DNA accumulates more changes over time. These early findings need more research to understand whether they have any biological importance.

We also looked at families where there were concerns about health conditions like birth defects, cancers, or other inherited diseases. Despite these concerns, we didn't find a clear connection between the genetic changes in the veterans and any health problems reported

in their children or grandchildren. This suggests that if there is any effect, it may be too small to detect with current methods, or it might not be linked to radiation exposure.

Conclusion

The findings of our study are reassuring for nuclear test veterans and their families. While some small changes were observed in a very small group of families, there is no strong evidence to support the idea that radiation exposure before conception caused genetic changes in the children of these veterans.

Background to the study

The harmful effects of ionising radiation exposure during pregnancy are well established. However, the potential heritable effects of parental radiation exposure before conception in humans remain uncertain and controversial [1-4]. Despite some non-significant trends, no epidemiological study has conclusively shown adverse health effects in the children of parents exposed to radiation prior to conception [1, 5, 6].

At the genetic level, some studies have reported an excess of DNA mutations in children of exposed parents [7-10], though others have found no such effect [11-13]. This includes the most recent whole genome sequencing analysis of family trios with parental exposure related to the Chernobyl accident [14, 15].

The International Commission for Radiological Protection (ICRP) estimates the hereditary risk in humans from parental radiation exposure to be 0.2% per Gray (Gy). However, this figure is derived from large-scale animal studies and is not supported by observed increases in hereditary disease in humans, suggesting the actual risk may be lower [16].

British nuclear test veterans represent a unique group of former military personnel who may have been exposed to ionising radiation during nuclear weapons testing in the 1950s and 1960s. Over the years, members of this community have voiced concerns about their health and the health of their descendants, which some believe may be linked to their participation in these tests [17].

Early epidemiological studies of mortality and cancer incidence among these veterans—conducted up to 1998—initially found limited evidence of adverse effects [18, 19]. However, after longer follow-up to 2017 this was revised to indicate a small but statistically significant increase in overall mortality (RR = 1.02; 90% CI 1.00–1.05; p = 0.04), with similar increases for both cancer and non-cancer illnesses [20].

No formal epidemiological studies have been conducted to assess the health of descendants of nuclear test veterans. This is partly due to limited evidence of health effects in the veterans themselves, and partly because national registries of birth outcomes were not established until decades after the nuclear tests took place. Nonetheless, anecdotal reports from nuclear test families have described adverse health outcomes in descendants at rates higher than in the general population [21]. While considered controversial by most mainstream scientists, such reports understandably raise concerns in veterans.

Sources and potential routes of radiation exposure

Between October 1952 and September 1958, the UK conducted 21 atmospheric nuclear tests of varying yield (measured in kilotons of TNT) at sites in Western and South Australia and the South Pacific. In addition, approximately 580 minor or experimental trials were conducted in South Australia between 1953 and 1963. These sites were decommissioned and cleaned up by 1967 [22, 23].

Potential sources of radiation exposure during these tests included immediate (prompt) exposure from the detonation itself, exposure to radioactive materials released during the

test, and longer-term exposure from distance fallout. Prompt exposure occurred within the first minute after detonation and consisted mainly of gamma rays and neutrons. The radiation dose from this exposure decreases rapidly with from the detonation point—dropping to 10–20 milligray (mGy) at 2.5 km and becoming negligible beyond 5 km [24].

During British tests, most personnel were located well outside the immediate blast zone: on beaches approximately 40 km away, on airfields about 32 km away, or aboard ships stationed outside the affected area. Nonetheless, several radioactive substances posed potential risks. These include activation products (formed when neutrons interact with nearby materials), fission products (generated from uranium or plutonium splitting), and residual nuclear fuel. While many radionuclides decay quickly, long-lived isotopes such as Strontium-90, Uranium-235/238 and Plutonium-239 can result in a sustained internal radiation dose over a person's lifetime [25-27].

Atmospheric detonations released significant quantities of radioactivity into the upper atmosphere. Although many bombs were deliberately exploded at high altitudes to reduce local fallout, factors like weather conditions and blast efficiency influenced how radioactive material dispersed and where it eventually settled. Fallout consisted of activation and fission products, as well as unconsumed uranium and plutonium. Its spread was affected by weather variables such as rainfall, wind speed and direction, and temperature gradients [28, 29].

External radiation exposure could occur when individuals walked over or worked in contaminated areas, flew through nuclear plumes to collect samples, or sailed through contaminated air or waters. While exposure generally ceased once individuals left the contaminated area, it could continue if radioactive particles were carried on clothing, equipment, or within enclosed vehicles. Internal exposure could occur through inhalation or ingestion of radioactive dust from contaminated materials, resulting in an internal dose that depends on the quantity inhaled, the radionuclide's half-life, and its emission characteristics [26].

Table 1. UK atmospheric nuclear tests and minor experimental trials in Australia and south pacific 1952-63

Site	Operation	Date	Purpose of Operation	Yield of Bomb (kt of TNT)
ATMOSPHERIC TESTS				
Monte Bello Islands	Hurricane	3/10/52	Weapon effects	25
Emu[1]	Totem 1	15/10/53	Weapon development	10
	Totem 2	27/10/53	Weapon development	8
Monte Bello Islands	Mosaic G1	16/05/56	Weapon development	15
	Mosaic G2	19/06/56	Weapon development	60
Maralinga[1]	Buffalo 1 (One Tree)	27/09/56	Test warhead	15
	Buffalo 2 (Marcoo)	4/10/56	Test warhead	1.5
	Buffalo 3 (Kite)	11/10/56	Gather data	3
	Buffalo 4 (Breakaway)	22/10/56	Test service weapon	10
Maldon Island	Grapple 1	15/05/57	Test thermonuclear weapons	300
	Grapple 2	31/05/57	Test thermonuclear weapons	720
	Grapple 3	19/06/57	Test thermonuclear weapons	200
Maralinga[1]	Antler 1 (Tadje)	14/09/57	Weapon development	0.93
	Antler 2 (Biak)	24/09/57	Weapon development	5.67
	Antler 3 (Taranak)	9/10/57	Weapon development	26.6
Christmas Island	Grapple X	8/09/57	Test thermonuclear weapons	1800
	Grapple Y	28/04/58	Test thermonuclear weapons	3000
	Grapple Z1	22/08/58	Test thermonuclear weapons	24
	Grapple Z2	2/09/58	Test thermonuclear weapons	1000
	Grapple Z3	11/09/58	Test thermonuclear weapons	800
	Grapple Z4	23/09/58	Test thermonuclear weapons	25
EXPERIMENTAL TRIALS				**Number of trials**
Maralinga[1]	Kittens[2]	1953–1961	Testing weapon components	99
	Tims	1955–1963	Testing weapon components	321
	Rats	1956–1960	Testing weapon components	125
	Vixen A	1959–1961	Dispersal by fire and explosion	31
	Vixen B	1960–1963	Effect of accidental detonation	12
	Ayres, Hercules, Brumby	1960-67	Clean up operations	

[1] South Australia

[2] The first Kittens trials in 1953 took place at Emu

According to Health Physics records [23], external radiation doses were officially recorded for only 21% of the full cohort of veterans and just 8% had a measurable (non-zero) dose. Of these, 1,635 participants received estimated doses between 0–50 millisievert (mSv) over the course of the test programme. Forty-four individuals were estimated to have received 50–100 mSv, and 36 veterans more than 100 mSv.

In total, 759 veterans were identified as members of “special groups,” including those with potentially higher exposure risks, such as the Buffalo Indoctrinee Force, RAF air sampling crews, personnel decontaminating aircraft, the HMS Diana crew (which sailed through a nuclear plume during Operation Mosaic), and the Target Response Group at Buffalo. In addition to support roles like construction and catering, some veterans performed tasks in contaminated areas days or even months after tests. These activities may not have been reflected in formal exposure classifications.

Purpose and Aims

The Genetic and Cytogenetic Family Trio (GCFT) Study was designed in response to long-standing concerns that veterans of the British nuclear testing programme may have received sufficient radiation exposure to cause genetic damage in themselves which could potentially be passed to their children, affecting the health of future generations. While not all genetic changes result in health problems, they can provide insight into an individual’s overall genomic health and potential disease risk.

The central purpose of the GCFT Study was to explore whether a heritable genetic legacy, associated with radiation exposure due to participation in British nuclear test operations, existed. To address this, we recruited family trios comprising a veteran, the child’s mother, and an adult child conceived after the veteran's final deployment to a nuclear test site. Veteran participants were selected based on their likelihood of exposure to ionising radiation and then matched by age, service branch, and rank with veterans who served in tropical regions but did not participate in nuclear testing.

The study set out to answer two primary questions:

1. Is there any chromosomal evidence of past radiation exposure in nuclear test veterans?

2. Is there evidence of genetic changes—either inherited or acquired—in the adult children of these veterans?

Overview of recruitment, sampling and analysis

Eligible nuclear test and control veterans were identified from the UK Nuclear Test Veteran (NTV) cohort [19, 23, 30, 31]. Public Health England (now UK Health Security Agency (HSA)), as custodians of the cohort, provided anonymised exposure data—including age, test participation, and “special group” status—for 5,818 test veterans and 6,101 controls who were under 82 years old, alive, and believed to be cancer-free.

To select the veterans with the highest likelihood of exposure, a long-list of 1,459 NT veterans were chosen if they were aged 80 or younger and had participated in two or more operations, including the GRAPPLE X, Y, and Z series or Maralinga tests, or were listed as special group members. An additional 42 veterans aged 82 or younger from specific high-exposure groups—such as the HMS Diana crew, Active Handling Flights, and Air Sampling Plume teams—were also included.

Using this list, NHS Digital provided contact details for the veterans' general practitioners (GPs) for 908 nuclear test and 3,796 control veterans, enabling the team to invite participants via their GP practice. All 908 nuclear test and 2,741 control GPs were contacted with the request to forward invitation packs. In total, 405 test veterans and 1,028 control veterans (matched by age, military branch, and deployment period) were known to be invited to participate (Figure 1).

Veterans who responded were screened via telephone to confirm eligibility and were then asked to provide written informed consent. Additional information was gathered through a structured questionnaire covering military service history and potential exposures to other clastogenic (DNA damaging) agents. Eligible veterans were asked to share study details with a child conceived after the nuclear test veteran's last deployment to a nuclear test site. If the couple had more than one child, the first child conceived post-deployment was prioritised to reduce time between exposure and conception and minimise selection bias [5]. Interested adult children were also screened and, with GP confirmation of eligibility, provided written consent. Participants were excluded if they had undergone chemotherapy or radiation therapy for any condition, as such treatments could lead to genetic damage which would make interpretation of the results difficult.

After consent, each family was sent a sample kit to collect whole blood. Blood was drawn by the participant's GP and shipped to Brunel University of London within 24 hours. Samples were processed under Human Tissue Authority guidelines. Lithium heparin samples were analysed cytogenetically at Brunel, while EDTA tubes were sent in batches to the University of Leicester for whole genome sequencing (WGS) [14, 32-35].

Most test veterans in the UK NTV cohort have no recorded dose as only a limited number were issued with film badges, mainly accounting for those identified in special groups and, no measurement for internal contamination took place. Accordingly, based on the questionnaire testimony and operation information drawn from the UK NTV cohort database and, blind to any results, the nuclear test veterans were assigned to a three-point rank for potential of internal/external exposure. Each case was *a priori* assumed to be in the lowest rank (exposure rank 1), and a higher rank allocated only if sufficient information was given to suggest a higher likelihood for radiation exposure. A defined role in a contaminated or forward area (e.g. aircraft sample retrieval/cleaning) undertaken more than once was considered a higher exposure potential, and here we distinguished between activities immediately and up to 3 months after the test where dose and dose rates would be expected to be highest (exposure rank 3) or, at any time from at least 3 months after the test (exposure rank 2). Geographical location of the test site was also considered relevant. For instance, the potential for a veteran working in a 'forward area' at Maralinga to be exposed

to both external and internal radiation was assumed to be higher than a veteran who witnessed an atmospheric test in the safety zone (~40 km from the blast) on Christmas Island. Thus, although this 'potential for exposure' ranking cannot be considered a substitute for recorded radiation dose, it was employed as a proxy from which sub-groups of the NT cohort could be defined.

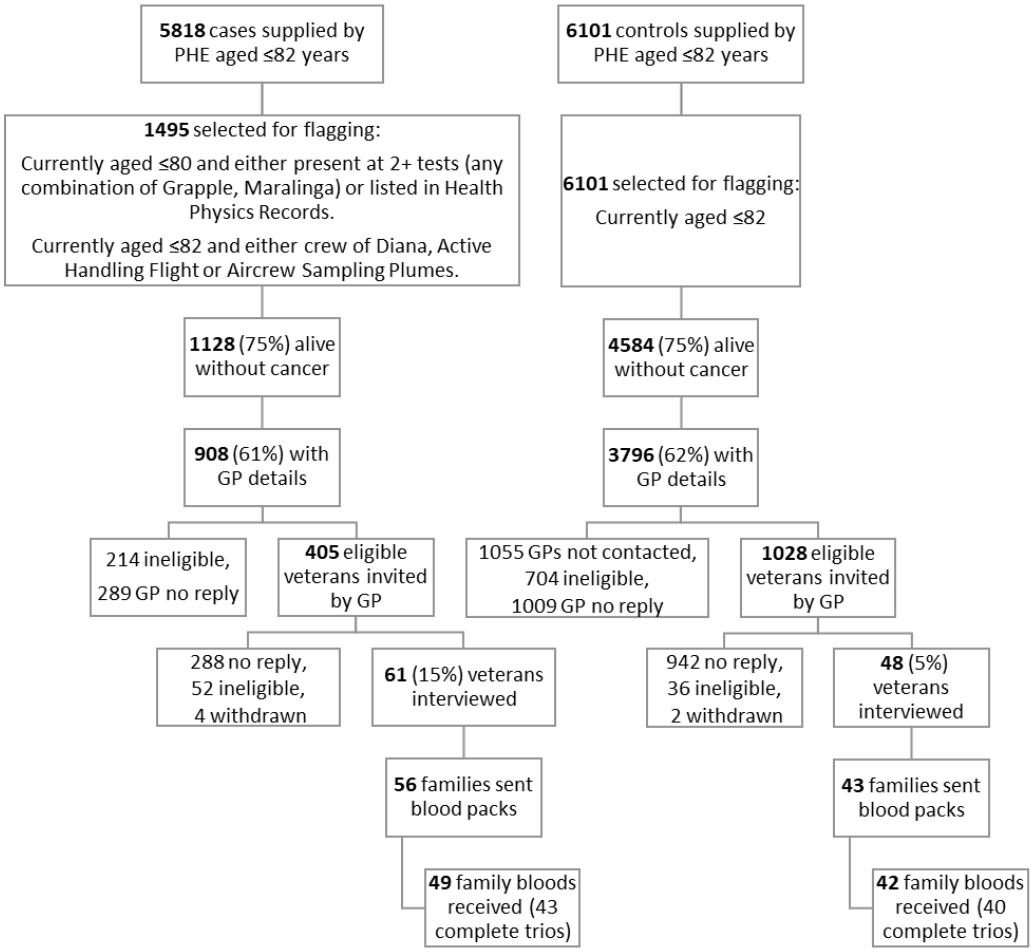

Figure 1: Study Flowchart: Cohort selection, invitation and response

Outcomes from study

Characteristics of cohort recruited

Blood samples were received from 91 families—49 nuclear test and 42 control—comprising veterans from the Army, Royal Air Force (RAF), and Royal Navy. Among the test veterans, 19 (39%) were categorised as having medium or high exposure potential (exposure ranks 2 or 3), including 13 previously identified as part of special groups or recorded in Health Physics documents.

Recruiting full family trios (veteran, child, child's mother) proved challenging due to the older age of the cohort, with veterans averaging around 80 years of age [36]. Data protection regulations required that initial contact be made via the participant's GP practice, which limited the ability to confirm whether the invitation had been received or to issue reminders. Most veterans did not respond (71% of nuclear test and 92% of control veterans), and the eligibility of non-responders is unknown.

The recruitment process involved multiple steps—eligibility confirmation, screening, consent from couples, followed by child recruitment—further reducing overall participation. Ultimately, 14% of nuclear test and 4% of control families submitted at least one blood sample. Recruitment ceased in March 2020 due to the COVID-19 pandemic, at which point samples had been received from 49 test and 42 control veterans (though 6 nuclear test and 2 control families were incomplete trios).

Participation Bias

Low participation rates may reflect self-selection bias. Nuclear test veterans who believed they or their families were affected by radiation may have been more motivated to participate. Indeed, 10 of the 49 nuclear test veterans (20%) reported at least one congenital abnormality among their children or grandchildren, compared with only 2 control families (5%)—a statistically significant difference ($p = 0.03$). This rate is also higher than the general population prevalence of approximately 2% [37].

While this may suggest a true increase in congenital abnormalities among descendants of nuclear test veterans, it may also reflect selection bias if families with health concerns were more likely to take part. Establishing an unbiased estimate would require systematic linkage of family health records for all veterans of the nuclear testing programme, which would be a significant undertaking.

A higher proportion of control veterans (43%, n = 18) reported occupational radiation exposure compared to nuclear test veterans (20%, n = 10). However, only two individuals in each group reported exposures likely to be relevant, and most occurred outside of military service. Other reported exposures included working with radar equipment (which can emit parasitic X-rays) or working near nuclear facilities/devices. The longer average service duration of control veterans may explain the increased reporting. Again, it could be that longer serving control veterans, or those exposed to radiation at other points in their careers may be more interested in our study and were more likely to take part.

Potential for exposure rank allocation

Only ~7% of the UK NTV cohort had recorded dose information yet testimony from veterans in the public domain highlighted concerns that exposure was not limited to just those issued with film badges. Accordingly, in this study, selection included those with no record of dose but whose potential for exposure was increased through attendance at multiple operations and special group status. These assignments were cross-referenced with interview data and operational records.

Potential for exposure rankings were determined before any cytogenetic results were available, making this a blinded and unbiased categorisation. Among nuclear test veterans aged ≤80, roughly one-third selected for flagging by NHS Digital belonged to a special group and/or present at 2 or more tests including Grapple or Maralinga. Despite this, 30 out of 49 test veterans who were recruited to participate in the study were categorised into the lowest potential for exposure rank (rank 1), as most recalled observing tests from designated safety zones. Higher potential for exposure rankings (ranks 2 and 3) was associated with service at Montebello or Maralinga sites or participation in high-risk roles, such as aircraft cleaning or sample recovery—activities that may have resulted in inhalation of radioactive particles from fallout.

Comparisons between cohorts and nuclear test sub-groups

Veteran chromosomal aberrations

Ionising radiation is known to cause DNA double-strand breaks (DSBs), a key lesion leading to structural chromosomal aberrations [38]. Fluorescence in situ hybridisation (FISH), a technique that uses fluorescent dyes to “paint” chromosomes, enables the detection of structural changes like reciprocal translocations and is widely validated for assessing past radiation exposure [39, 40].

Reciprocal translocations, which are simple exchanges between two chromosomes, are particularly informative as they are stable in cells over time and can persist in the body for decades following exposure [41-45]. However, these translocations also accumulate naturally with age due to other factors. To provide a more comprehensive view, we used multiplex-FISH (M-FISH), which uniquely paints each chromosome pair a different colour, allowing all 24-pairs of human chromosomes to be identified. This allows more complex, multi-chromosome rearrangement patterns to be seen [46, 47].

Complex chromosomal aberrations, involving three or more breaks across two or more chromosomes, are typically induced by low doses of high-LET (Linear Energy Transfer) radiation [48]. This type of radiation, which includes alpha-particles, deposits a large amount of energy in a very short distance which is known to cause dense localised DNA damage which is difficult to repair. The frequency and type of chromosome aberrations observed by M-FISH are thus potentially informative of radiation exposure, dose and radiation type.

No difference in chromosome aberration frequencies between control and nuclear test veteran cohorts

We analysed blood samples from 48 nuclear test and 38 control using M-FISH, with a total of 9,379 and 7,698 metaphase cells examined respectively. The number of metaphase cells per sample ranged from 78 to 390 (median=196); 18 samples had less than 150 cells analysed, with no significant differences detected between the cohorts.

Both cohorts exhibited stable and unstable simple and complex aberrations. However, there were no statistically significant differences in the frequency of any aberration type between nuclear test and control veterans ($p > 0.2$ unless otherwise noted) (Figure 2 A-E).

The mean translocation frequencies were 1.461 ± 0.166 per 100 cells in the nuclear test cohort and 1.416 ± 0.234 in controls, aligning with age-matched expectations [49, 50]. Of the seven (representing three control and four nuclear test) veterans who did show an excess occurrence of translocations, six were smokers [50].

A comparison with previous studies offers context. Wahab et al, 2008, who assayed New Zealand (NZ) nuclear test veterans who had been on-board ships at the time of atmospheric tests together with an age-matched land-based control group using M-FISH, showed a control frequency of 1.005 ± 0.886 /100 cells consistent with the NZ population being ~20 years younger than those studied here [51]. However, the 3-fold increase in translocations (2.938 ± 1.752/100 cells) reported for NZ nuclear test veterans, is markedly different to what we see, indeed the frequency of translocations in just those British nuclear test veterans who were on-board ships in this study, although small (N=8), is slightly lower than the averages for all exposure ranks combined (0.816 ± 0.471 and 1.285 ± 0.284 translocations/100 cells for those on-board ships allocated to exposure ranks 1 and 3 respectively). Of the potentially relevant confounders, only smoking was reported by Wahab et al., meaning we cannot directly compare confounder profiles between both studies. It is noted from a technical perspective however that, in the Wahab et al. study, blood was cultured for longer than is standard and further, that a higher frequency of Robertsonian translocations and incomplete (one-way) reciprocal translocations compared to what we observed, were reported [51]. Instead, our findings align more closely with studies of French nuclear veterans [44] where no significant aberration frequency differences were reported.

Collectively, this suggests that—within the detection limits of this study—the sampled British nuclear veterans did not experience radiation exposure sufficient to cause long-lasting chromosomal changes detectable in peripheral blood lymphocytes by M-FISH.

This provides reassurance to veterans and their families, as the chromosomal evidence does not support widespread significant radiation exposure among the nuclear test cohort. In reporting this, we do not preclude the possibility that our findings reflect the sampling of nuclear test veterans who remained alive into their 80s, nor does it infer the same or different outcome would have been seen if veterans who have since passed away had been examined.

Higher frequencies of complex chromosome aberrations in a small group of nuclear test veterans

Due to the absence of formal dose records for most veterans and as stated earlier, nuclear test veterans were assigned to exposure ranks based on a three-point scale, reflecting their potential for radiation exposure. Veterans allocated to exposure rank 3 were considered to have the highest likelihood of radiation exposure. Geographical site was also a factor; for example, veterans stationed in forward areas at Maralinga were assumed to have higher internal and external exposure risks than those observing tests from safety zones on Christmas Island [52].

Thus, sub-groups of the nuclear test cohort were defined to assess whether aberration frequencies varied based on exposure potential or geographical location. Overall, no

statistically significant differences for chromosome aberration types were observed (Figure 2). However, although the numbers of veterans in each sub-group are small (meaning the statistical detection limit for identifying potential group differences were too high), nuclear test veterans classified in exposure rank 3 and stationed either onboard ships or at Maralinga exhibited higher average frequencies of complex aberrations compared to the nuclear test (0.299 ± 0.075 per 100 cells) cohort overall (Figure 2C).

Specifically, nuclear test veteran's onboard ships assigned to exposure rank 3 (N=4; 3 out of the 4 personnel were on HMS Diana) had higher average frequencies of complex aberration of 0.350 per 100 cells. Veterans at Maralinga with the same higher exposure rank (N=5) exhibited a higher frequency of 0.803 ± 0.191 per 100 cells. These subgroups of veterans were also associated with increased unstable aberrations, including elevated dicentric equivalents compared to all other subgroups, all nuclear test veteran ranks combined and controls. The presence of unstable chromosome elements suggests their formation to be relatively recent events within the lifespan of the cell.

The average background level of complex aberrations in the general population aged 70–80 years has been reported as 0.128–0.193 per 100 cells [53]. However, the frequencies observed in the sub-groups highlighted are more consistent with levels reported in nuclear workers with internalised plutonium exposure, where complex aberrations range from 0.200 to 1.00 per 100 cells, and even higher in heavily exposed individuals [43, 54-56]. Further, complex aberrations of the pattern, size and complexity (number of chromosomes and breaks involved) as detected here are characteristic of induced aberrations seen after exposure to low doses of high-LET radiation, or high doses of low-LET radiation [48, 57, 58], rather than from any ongoing instability to the genome.

Plutonium-239, a long-lived alpha-emitter, is a known component of nuclear fallout. If inhaled, it accumulates in the liver and skeleton, with a small proportion excreted in urine over time [59]. Plutonium-239 is also encountered in industry, however, varying isotopic ratios of $^{240}Pu/^{239}Pu$ can be used to establish the potential source of Pu as the isotope in weapons is primarily ^{239}Pu. The potential for detecting $^{240}Pu/^{239}Pu$ in the urine of nuclear test veterans after this length of time (~60 years) was limited. However, a pilot study was undertaken to analyse urine samples from 20 veterans (nuclear test and control) using accelerator mass spectrometry to detect isotopes of plutonium. Initial analysis shows two veterans to show detectable levels of plutonium both with large uncertainties, one of which had an elevated complex aberration frequency and had reported working in the nuclear industry. No other of the 20 urine samples had detectable plutonium levels (Jerome et al. in prep, personal communication).

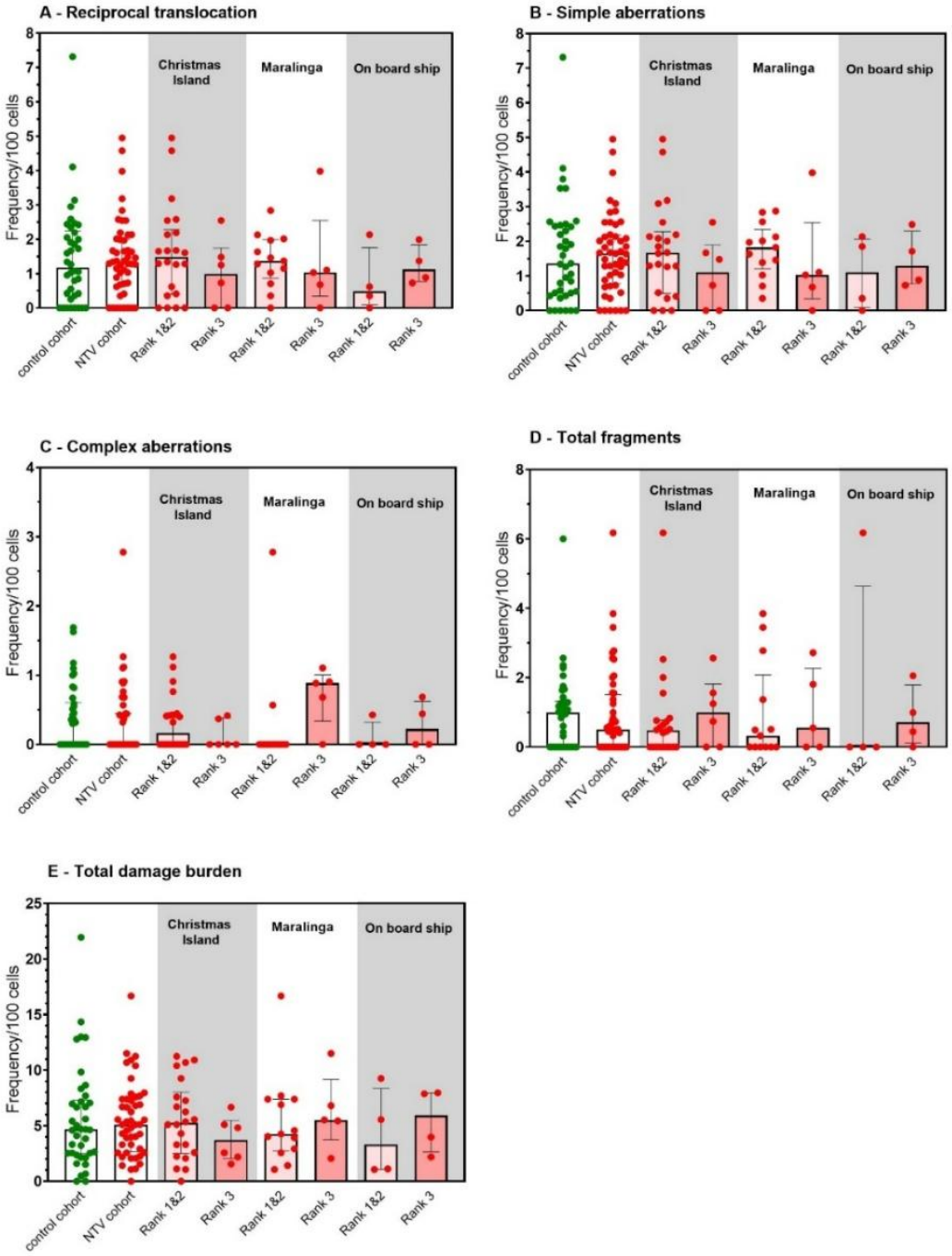

Figure 2: Structural chromosome aberrations observed in control and test veteran cohorts. Frequency of chromosome aberration types per 100 cells reported for control and nuclear test cohorts and, for nuclear test subgroups based upon geographic location (Christmas island, Maralinga range or on board a ship at time of test) and, potential for radiation exposure ranking (allocated blind to cytogenetic data as lower [1], medium [2] or higher [3] potential) [52]. A. Total reciprocal translocations (complete and incomplete types), B. simple exchanges (total reciprocal translocations, Robertsonian translocations and total dicentric chromosomes), C. complex chromosome exchanges (complete and incomplete types), D. total fragments (total associated with complex, dicentric, ring or break-only aberrations), E. Total damage burden (total number of chromosome breaks irrespective of aberration type). The box-whisker plots show the values for each veteran (dots) together with median values (bar) and the 25 to 75% interquartile range (whiskers).

Thus, we cannot corroborate from this urine analysis that the origin of the complex chromosome aberrations observed in the small group of nuclear test veterans are associated with ongoing exposure to internalised radionuclide contamination. However, it is considered likely given what we understand about the mechanistic formation of complex aberrations from α-emitters [34, 48] and the potential for exposure in these exposure rank 3 groups [52]. Indeed, the MoD state that the potential for internalised radionuclide exposure may have arisen in Maralinga and HMS Diana. Wahab et al [51, 60] also reported an excess of 'very complex' chromosome aberrations in NZ nuclear test veterans' that were not seen in the control group.

Constitutional chromosomal abnormalities in adult children

Constitutional aberrations are defined as alterations in the number or structure of chromosomes present in all cells of an individual at birth and which are typically associated with a distinct set of clinical features. These abnormalities can arise spontaneously or be inherited from one or both parents. This part of the study aimed to investigate whether children born to nuclear test veterans displayed any constitutional chromosomal abnormalities.

No constitutional chromosomal abnormalities detected in a cohort of nuclear test children

A total of 76 samples were stained for G-band analysis, which is a technique to look for microscopically visible changes to identify any constitutional chromosomal aberrations, if present. The majority of cells analysed were between 350-550 banding resolution [61]. 10 samples either did not culture or were technical fails.

We examined for but found no evidence of any constitutional chromosomal abnormalities in a sample of adult children born to nuclear test veterans. All individuals exhibited apparently normal karyotypes—46,XX or 46,XY—including those from families who self-reported adverse health effects. Cells with the highest possible G-band resolution were selected for analysis; however, it is acknowledged that most constitutional abnormalities observed in individuals who survive to adulthood are likely to involve small structural alterations or balanced exchanges, which may be difficult to detect through standard karyotyping. Thus, we re-examined whole-genome sequencing (WGS) germline data [62] but again found no evidence of genetic variants at loci potentially relevant to the conditions reported at the time of the interviews [52]. A key criterion of the GCFT study was to recruit the first-born child conceived after the veteran's final nuclear test site deployment, aiming to minimise the interval between exposure and conception (time is thought to be one explanation for the differences seen between species where unlike human data, animal data shows strong evidence for radiation effects across the generations) [5], and reduce bias. However, consequently, most reported health conditions affected siblings of the sampled child. Nonetheless, no constitutional abnormalities were observed in any of the NT cohort, including those individuals for whom health concerns were reported.

Genomic instability in somatic cells of adult children

Genomic instability refers to an increased tendency to acquire diverse genomic alterations including DNA mutations, chromosomal aberrations, epigenetic changes and dysregulated gene expression. Cytogenetically, this may manifest as non (or sub)-clonal stable and unstable chromosomal changes—such as reciprocal translocations or dicentrics—as well as chromosome breaks, fragments, and chromatid-type (damage to just one strand of a chromosome) aberrations [63, 64].

Animal studies by Dubrova and colleagues demonstrated that radiation or chemical exposure to the germline can lead to increased frequencies of mutations and chromosomal aberrations in offspring, a phenomenon termed transgenerational genomic instability (TGGI) [7, 65]. However, evidence for radiation-induced TGGI in humans remains inconclusive. This part of the study aimed to investigate whether children born to nuclear test veterans displayed any cytogenetic evidence of genomic instability.

Genomic instability in a small sub-set of children born to nuclear test veterans

Blood samples were cultured to obtain first-division metaphase cells, which were Giemsa-stained for brightfield analysis. The aim was to detect aneuploid (an incorrect number of chromosomes) and unstable structural chromosomal aberrations, as well as chromatid-type aberrations indicative of a genomic instability phenotype. A total of 5,897 and 3,759 cells were scored from 33 nuclear test and 26 control adult children, respectively. An abnormal cell was defined as one containing at least one structural or numerical aberration.

After adjusting for confounding variables, there were no statistically significant differences in the overall frequencies of unstable structural chromosome aberrations (1.61 ± 0.24 and 1.63 ± 0.28 per 100 cells), chromatid aberrations (4.68 ± 0.69 and 4.36 ± 0.62 per 100 cells), or aneuploid cells (8.40 ± 0.69 and 6.42 ± 0.99 per 100 cells) between nuclear test and control cohorts, respectively. This lack of difference held true for cases where aneuploidy was accompanied by structural chromosome or chromatid aberrations. The only notable difference was observed in one subtype of chromosome aberration—chromosome discontinuities—which were nearly three times more frequent in children born to nuclear test veterans (0.40 ± 0.09 per 100 cells) than in controls (0.15 ± 0.08 per 100 cells).

When the analysis was stratified by nuclear test veteran father subgroups, this increase was only statistically significant among adult children of veterans who served at Christmas Island with low to medium exposure potential (N=12; ranks 1 and 2), where the frequency of discontinuities was 0.54 ± 0.19 per 100 cells. This was higher than the average across all nuclear test children (0.40 ± 0.09). Children of rank 3 veterans from Christmas Island (N=5) showed a similar frequency (0.50 ± 0.32), but the small sample size likely prevented statistical significance.

A separate trend was observed in a small group (N=4) of adult children of veterans who had served onboard ships during the tests (exposure rank 3). This group showed elevated chromatid aberrations—both in complete (7.8 ± 4.01/100 cells) and aneuploid (1.75 ± 1.18/100 cells) cells —which may reflect ongoing genomic instability. While these

differences were statistically significant ($p = 0.02$ logistic regression), they did not remain robust under sensitivity analyses. Accordingly, these observations should be interpreted with caution and warrant validation in larger cohorts.

Thus, we find only limited evidence of genomic instability, and only within the subgroup of adult children born to Christmas Island veterans (chromosome discontinuities i.e., chromosome breaks) and an increased frequency of chromatid aberrations—both in complete and aneuploid cells—in adult children of veterans who had served on ships (exposure rank 3).

Although these findings were adjusted for potential confounders, it is important to note that the information available for adult children was limited. Information collected via telephone interviews included the number of diagnostic scans (X-rays, CT scans), but not occupational exposure, smoking history, or lifestyle factors. Additionally, scan locations (i.e., anatomical sites) were not recorded.

The confounder analysis identified a history of CT scans as a strong predictor of elevated chromosomal aberration frequencies. Interestingly, a higher number of X-rays was associated with fewer chromatid-type aberrations, an inverse relationship that aligns with the established understanding that chromosome-type, rather than chromatid-type, aberrations are more typically induced by ionising radiation from diagnostic imaging. This may explain the increased frequency of chromosome discontinuities in adult children of Christmas Island veterans.

In contrast, the weakly significant elevation in chromatid-type aberrations observed in the small group of adult children of ship-based veterans is consistent with a phenotype of ongoing genomic instability. However, due to the very limited sample size in this subgroup ($N = 4$), this finding cannot be generalised to all offspring of ship-based nuclear veterans.

Germline mutations

De novo mutations (DNMs) such as single nucleotide variants (SNVs), insertions/deletions (INDELs) and structural variants (SVs) are new genetic changes (mutations) that appear for the first time in a child but are not present in either parent. These mutations can result from environmental exposures, errors in DNA replication, or random events and for most, have no effect on health with current estimates predicting ~ 50 - 100 new mutations per individual per generation [66-68]. Whole genome sequencing (WGS) allows for comprehensive identification and analysis of DNMs across the entire genome.

Exposure to ionising radiation is known to increase the mutation burden with evidence from animal studies showing elevated DNMs following parental exposure to acute high doses of radiation [5]. For instance, highly significant increases in the incidence of SVs, INDELs and clustered DNMs (e.g. multiple SNVs within a few base-pairs of each other) have been reported in mice exposed to 3 Gy ionising radiation [69]. In humans, the data is mixed with reports showing significant elevations for families inhabiting the heavily polluted areas of Belarus and Ukraine following the Chernobyl accident and in the vicinity of the Semipalatinsk nuclear test site in Kazakhstan [9, 70, 71]. However, no such observations were seen by

whole genome sequence analysis of another group of parents exposed after Chernobyl [14, 15].

This part of the study used WGS to examine blood-derived DNA from 60 family trios (30 nuclear test and 30 control) to determine whether nuclear test veterans exhibited a higher mutation burden in their germline. Sequencing was performed to a high depth (average >35x coverage) to ensure reliable detection of de novo SNVs, INDELs and SVs. Variant calling was conducted using standard, validated bioinformatics pipelines. DNMs were identified by comparing the genomes of children with those of their biological parents, and all candidate mutations underwent strict quality control filtering to remove artefacts.

No evidence of increased mutations in the germline of a cohort of nuclear test veterans

Overall, we found the frequency of DNMs in nuclear test and control veteran families to be comparable with that previously reported for general populations. Additionally, the occurrence of DNMs increased with increasing parental age at the time of conception, consistent with expectations. A comparison of the frequency, type or distribution of mutations between the family cohorts showed no statistically significant differences in the amount or type of DNM (Figure 3). Overall, we found no significant association to any variable indicating any impact on germline mutation burden for any of the mutation endpoints in the nuclear test germline. These results align with other large-scale studies investigating potential heritable genomic effects of parental radiation exposure, such as those involving Chernobyl clean-up workers [14].

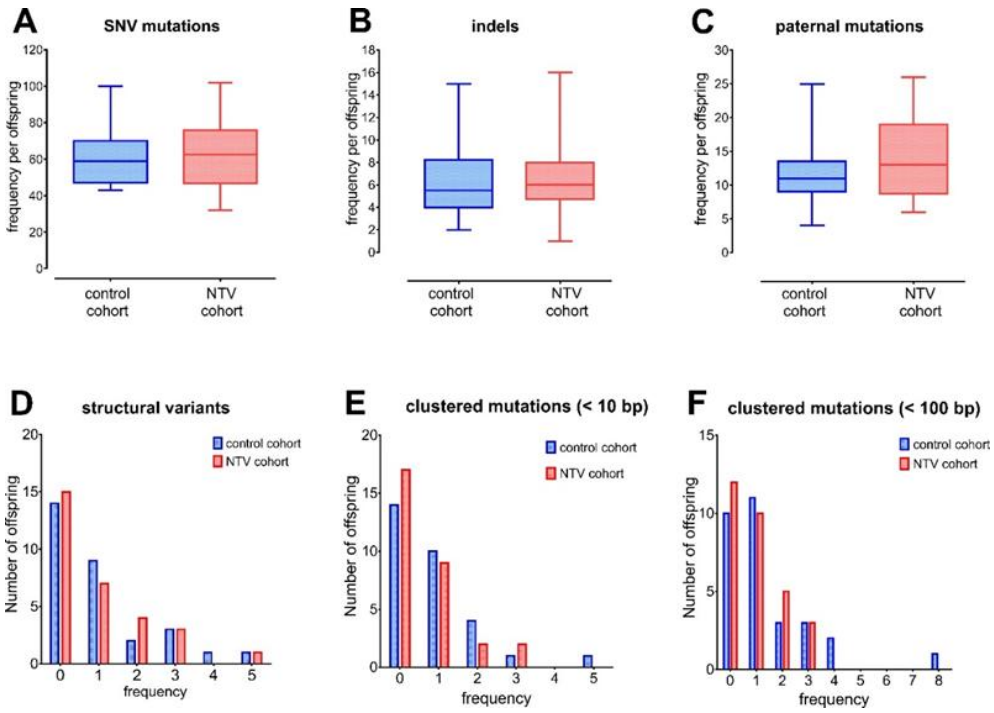

Figure 3: The frequency of *de novo* mutations per offspring in controls and NTV families. [A] SNV mutations; [B] indels; [C] paternal mutations; [D] structural variants; [E] clustered mutations (< 10 bp); [F] clustered mutations (< 100 bp). 95% confidence intervals are shown.

Over-representation of mutation signature SBS16 in small group of control and nuclear test families.

Single base substitution (SBS) signatures, along with other mutational signatures, represent identifiable patterns of DNA changes that are increasingly recognised across various cancer types. In non-cancerous cells, mutational signatures are like 'fingerprints' of damage left on DNA whereby different causes (e.g., UV light, tobacco smoke) leave distinct patterns, or signatures.

We compared our findings to a large database (COSMIC) of known signatures. For this, the total of 3,719 de novo germline single nucleotide variants (SNVs) detected in both control and nuclear test families were analysed using the COSMIC v3.2 database (March 2021). These mutational signatures are classified based on six substitution types—C>A, C>G, C>T, T>A, T>C, and T>G—as well as the immediate flanking nucleotides [72].

Two statistical approaches were applied. First, the total number of SNVs was analysed (1,851 in the control cohort and 1,868 in the nuclear test cohort). Eight SBS signatures were found to differ significantly between the cohorts, with SBS16 showing the largest difference (control: 432; nuclear test: 569; $p < 0.01$) (Figure 4A).

Second, we assessed whether these differences remained when considering the average number of SNVs per family (offspring) in each cohort. For this, only offspring from families with at least 10 individuals carrying one or more SNVs were included. This analysis identified SBS3, SBS12, and SBS16, though neither parametric nor non-parametric statistical tests confirmed significant average differences between cohorts (Figure 4B).

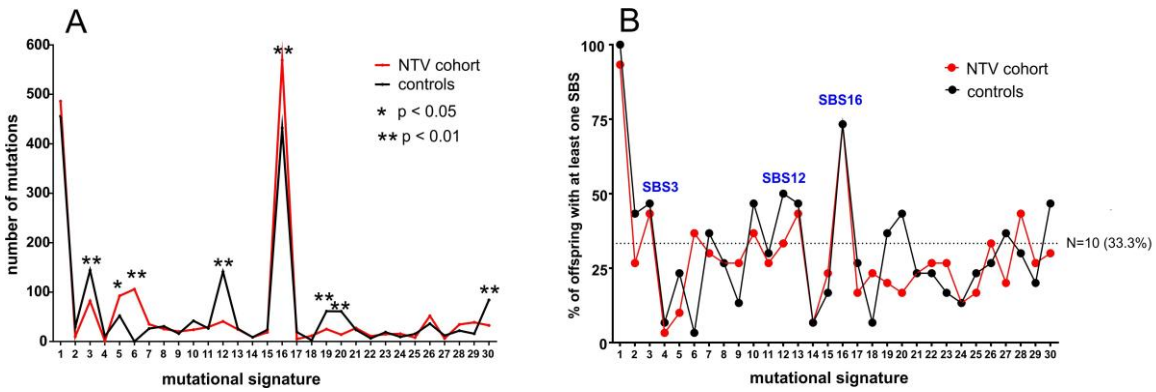

Figure 4: The spectra of mutation signatures in controls and nuclear test families. [A] Frequency of SBS mutations per signature. Data were fitted to the COSMIC v3.2 database with signature fitting distributing each mutation to one (and only one) signature. [B]. Families (offspring) with at least one mutation per signature. Statistical difference judged on a signature-to-signature basis (Chi square test, multiple p-value adjustment by the Holm method).

The discrepancy regarding SBS16 between the two analytical methods appears to stem from a subset of families. Specifically, six of the eight highest SBS16 counts were observed

in nuclear test veteran families (Figure 5). This suggests that a small group of nuclear test families likely accounts for the overall significant difference in SBS16, but this effect diminishes when averaged per individual. Therefore, it cannot be ruled out with sufficient confidence that this small group represents a random finding. That said, the six nuclear test families with the highest SBS16 counts did not show any associations with known confounding variables, including child age at sampling, paternal age at conception, history of paternal occupational radiation or chemical exposure, medical imaging, alcohol or smoking. Further, four involved veterans classified as having the highest potential for radiation exposure ranking (rank 3; includes two with a recorded dose of 0.4 and 1.4 mSv).

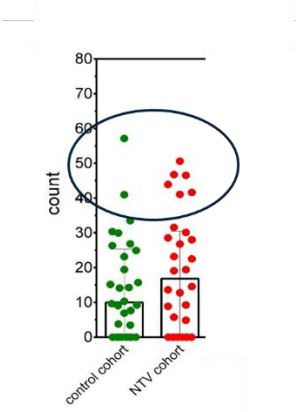

Figure 5: Total number of SNVs allocated to mutation signature SBS16. The high-SBS16 sub-group (N=8) was determined as those with >40 SNV mutations meaning the high-SBS16 subgroup comprised 2 control and 6 NT families.

Summary of findings

This report has presented a series of comparisons between control families, nuclear test families, and subgroups within the nuclear test cohort. Overall, the findings are reassuring. For the vast majority of nuclear test veterans, we found no chromosomal evidence of radiation exposure. Likewise, among their children, we observed no evidence of constitutional chromosomal abnormalities, elevated genomic instability in somatic cells, or increases in the number or types of germline mutations.

However, a small number of families showed elevated average frequencies in specific areas:

1. **Complex Chromosome Aberrations:** Potential indicators of long-lived internalised contamination were observed in nuclear test veterans (exposure rank 3) who had served at Maralinga (N = 5) and onboard ships (N = 4).
2. **Chromatid Aberrations:** A weak, non-robust statistical trend suggestive of genomic instability was found in adult children of nuclear test veterans who had served onboard ships (N = 4; veteran father's exposure rank 3).
3. **Over-representation of Germline Mutations Associated with Signature SBS16:** Identified in a small number of families across both control and nuclear cohorts (N = 8; 2 control and 6 nuclear test).

These specific findings will be examined in greater detail in the next section.

Intergenerational Effects

The family trio design of the GCFT study allowed for the quantification of germline mutation frequencies using whole genome sequencing. This design also enabled an investigation into potential intergenerational relationships in genetic burden within families. Specifically, matched veteran father–child datasets (in which both individuals had undergone analysis) were used to examine whether the father's chromosomal aberration burden was associated with the frequency of de novo germline mutations or somatic aberrations in their children.

Veteran Chromosomal Aberrations and Germline Signature SBS16

All families where both M-FISH data from veteran fathers and WGS germline data was available was used for this analysis (30 nuclear test and 28 control families). First, a comparison between the nuclear test and control cohorts revealed no significant associations between the father's total chromosomal damage or complex aberration burden and the number of germline mutations in the child. However, a different trend emerged when the analysis focused on just those families previously identified to have a high number of germline mutations associated with mutation signature SBS16 (the high-SBS16 group, Figure 5). Here, a weak association was observed between the presence of SBS16 in the germline and increased complex chromosomal aberrations in veteran fathers (borderline trend of p=0.054 for Wilcoxon rank-sum test and statistically significant p=0.032 for negative binomial regression).

Table 2 summarises this relationship, showing that in the high-SBS16 group, the frequency of complex aberrations in veteran fathers was 0.686 ± 0.315 per 100 cells, and the overall chromosomal damage burden was 7.546 ± 1.82 per 100 cells—both higher than in any other cohort or sub-group. Additionally, the average total number of de novo germline mutations (88.5) and single nucleotide variants (SNVs) (79) in this sub-group were also elevated.

These findings suggest a potential relationship between the enrichment of mutation signature SBS16 in the germline and complex chromosomal aberrations in veteran fathers, which, as discussed earlier, may be indicative of long-lived internalised α-emitting radionuclide exposure. This raises the possibility that SBS16 could reflect the molecular processing of radiation-induced DNA damage and could, therefore, serve as a candidate transgenerational biomarker of paternal radiation exposure. This is interpretation is complicated however by the presence of two control families within the high-SBS16 subgroup.

Table 2: Summary of chromosome aberration burden in veterans and de novo germline mutations.

	Veteran M-FISH data Frequency/100 cells (number)				Germline mutation frequency/adult child					
	Cells (stable)	Complex	Total damage burden	Translocation equivalent in stable cells	Total	SNV	InDel	SV	Cluster (10bp /100bp)	SBS16
SBS16[1]										
Families with >40 SNV mutations allocated to SBS16 (N=8)	1312 (1288)	0.686±0.315[2] (9)	7.546±1.82 (99)	2.562±0.717 (33)	88.5	79	8.1	1.4	0.9/1.4	46.3
Families with <40 SNV mutations allocated to SBS16 (N=50)	9885 (9772)	0.303±0.061 (30)	5.53±0.573 (547)	1.770±0.265 (175)	67	59.76	6.26	0.98	0.8/1.2	12.5
Families who self-reported health effect in offspring[3]										
Families reporting effect (N=16)	3355 (3318)	0.268±0.107 (9)	4.978±0.731 (167)	1.567±0.299 (52)	71.5	62.44	7.75	1.3	0.8/1.4	14.5
None (N=42)	7844 (7742)	0.382±0.089 (30)	6.107±0.711 (479)	2.015±0.320 (156)	69.4	62.4	6.05	0.93	0.8/1.2	18.2
Veterans allocated to Rank 3[3]										
Rank 3 (N=11)	2037 (2005)	0.540±0.156 (11)	6.284±0.900 (128)	1.546±0.535 (31)	70.1	62.2	6.9	1	0.6/1.1	24.3
Ranks 1+2 (N=19)	3525 (3493)	0.312±0.163 (11)	5.702±0.952 (201)	2.262±0.430 (79)	69.5	62.3	6.21	0.95	0.63/0.89	16.05
Rank 0 (controls) (N=28)	5637 (5562)	0.302±0.085 (17)	5.624±0.913 (317)	1.762±0.370 (98)	70.3	62.6	6.57	1.11	0.93/1.5	15.2
Ranks 0,1+2 (M=47)	9160 (9055)	0.306±0.083 (28)	5.655±0.200 (518)	1.932±0.279 (177)	69.9	62.5	6.43	1.04	0.81/1.3	15.5

Includes all families (nuclear test and control combined) where both veterans father M-FISH data and adult child's whole genome sequence data [62], were available.[1] Moorhouse et al [62], [2]statistical significance for difference: p=0.054 (Wilcoxon rank-sum/Kruskal-Wallis test) and p=0.032 (Negative binomial regression) [73], [3] Rake et al [52].

Child Genomic Instability and Germline Signature SBS16

We also examined families in which both M-FISH data from veteran fathers and Giemsa-stained chromosomal data from adult children were available (24 control and 33 nuclear test families). The focus was on unstable chromosomal aberrations in veteran fathers such as dicentric equivalents and chromosome fragments resulting from exchange-type aberrations. Chromatid aberrations were quantified from DAPI-stained metaphase spreads obtained from the M-FISH images.

When matched veteran father–adult child pairs were grouped by cohort (nuclear test or control), a non-significant upward trend was observed between paternal unstable aberration burden and the frequency of both unstable chromosome-type and chromatid-type aberrations in adult children from nuclear test families (Figure 6). No such trend was observed in control families.

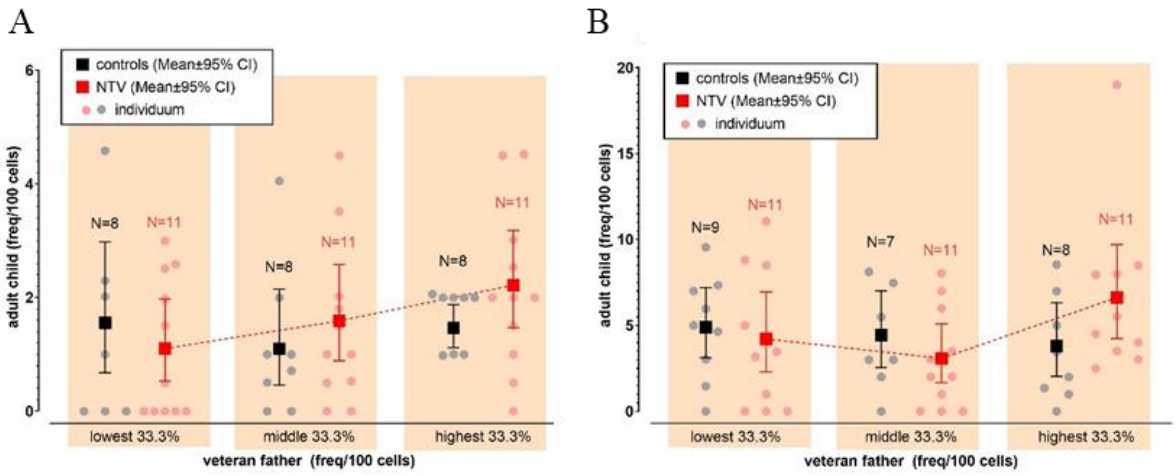

Figure 6: Association between veteran father and adult child aberration frequencies. Analysis was carried out for all veteran father-adult child samples where both veterans M-FISH data [73] and adult child Giemsa solid stained data [74] was available (24 control and 33 nuclear test). (A) Unstable chromosome aberrations and (B) chromatid aberrations. For (A) veteran fathers, unstable chromosome aberrations represent the total of the dicentric equivalent plus all fragments from simple, complex or breaks as detected by M-FISH while (B) chromatid aberrations were determined from the DAPI stained metaphase for each cell [73]. For both (A) and (B) veteran fathers' frequencies are categorised into tertiles, representing the lowest, medium, and highest thirds (each comprising approximately 33% of the data). For the adult children, the total aberration frequencies shown with mean values and 95% confidence intervals (CI) estimated using logistic regression model, accounting for overdispersion using the Williams method.

To investigate this further across cohort subgroups, a statistical modelling approach was applied. This revealed a significant positive trend between paternal chromosomal aberrations and increased chromatid aberrations in their adult children. Specifically, higher aberration frequencies in veteran fathers were linked with higher predicted frequencies in their children. Notably, this was trend was improved (p=0.02) within the small group of

families defined as high-SBS16 compared to the low-SBS16 group (Table 3). These findings suggest that the observed trend may be driven by the high-SBS16 subgroup, raising the possibility of a relationship between enrichment of mutation pattern SBS16, or an unidentified factor co-occurring in these families, and transgenerational genomic instability.

Self-Reported Family Health Outcomes

During the recruitment interview, both control and nuclear test veterans were asked whether they were aware of any birth defects, genetic disorders, inherited diseases, or cancers affecting their children or grandchildren [52]. After accounting for those with evidence of being familial and blind to cohort status, reported health conditions were categorised as congenital, cancer, or non-cancer. A significantly higher proportion of nuclear test families reported congenital abnormalities in their children or grandchildren compared to controls. This likely reflects heightened concern among nuclear test veterans, which may have influenced participation in the GCFT study.

Specifically, one-fifth of nuclear test veterans in our cohort reported at least one child or grandchild with a congenital abnormality—including two stillbirths—compared to a much smaller number reported by control families (Fisher's Exact $p = 0.03$). Additionally, three of the eight nuclear test and control families in the high-SBS16 subgroup self-reported a congenital condition within the family. No significant differences were found in reported cancer or non-cancer diseases among children (mean age: 53 years) between the nuclear test and control groups (Fisher's Exact $p = 0.19$ and $p = 0.6$, respectively).

Of these health concerns, significantly more were reported by veterans allocated to exposure rank 3 (across all geographic locations) than in other ranks. Specifically, health concerns were reported by 0.104 (5/48 families) in rank 0 (control), 0.31 (11/35 families) in ranks 1+2 combined, and 0.429 (6/14 families) in rank 3 ($p < 0.1$).

Among families reporting at least one health condition, only one control family and six nuclear test families provided blood samples from the affected child, in accordance with the protocol designed to minimise the interval between exposure and conception. Despite this, we found no evidence of an elevated chromosomal aberration burden in veteran fathers compared to families without reported concerns. Furthermore, we found no statistically significant relationship between paternal chromosomal aberration burden, germline mutation frequency, genomic instability, and self-reported adverse health outcomes in children or grandchildren (Table 3).

Table 3: Summary of unstable aberrations in veteran fathers and their adult children, stratified by family groups of interest

	Veteran father (frequency/100 cells)[1]					Adult child (frequency/100 cells)[2]			Germline SNV mutations
Cohort	Cells	Dicentric equivalent	Total fragments	Total unstable chromosome	Total chromatid	Cells	Total unstable chromosome	Total chromatid	SBS16[3]
SBS16[1]									
Families with >40 SNV mutations allocated to SBS16 (n=8)	1312	0.50±0.34	2.11±0.64	2.61±0.74	2.07±0.57	1617	1.88±0.47	7.75±1.97*	46.06
Families with <40 SNV mutations allocated to SBS16 (n=49)	9849	0.34±0.07	0.95±0.18	1.29±0.23	1.03±0.17	7749	1.44±0.19	3.89±0.42**	12.22
Families who self-reported health effects in their offspring[4]									
None (N=40)	7707	0.38±0.09	1.16±0.23	1.54±0.28	1.10±0.19	6286	1.56±0.20	4.89±0.46**	19.49
Effect (N=17)	3454	0.31±0.13	1.01±0.28	1.32±0.40	1.36±0.37	3080	1.37±0.37	3.37±1.14*	14.77
Congenital (N=10)	2073	0.39±0.17	1.18±0.38	1.57±0.53	1.60±0.43	1777	1.68±0.54	3.71±1.81	21.02
Non-cancer (N=52)	759	0.39±0.08	1.17±0.19	1.57±0.24	1.13±0.17	902	1.01±0.18	4.46±0.51**	4.62
Cancer (N=5)	1231	0	0.52±0.40	0.52±0.40	1.61±0.92	899	1.32±0.53	4.14±1.41	6.96

Includes all families for which both M-FISH data from the veteran fathers [1][73] and solid stain data from their adult children [2] [74] were available. Mean ± SEM frequency of aberrations per cell (calculated where N > 4, using participant as the statistical unit). [3] Number of germline mutations assigned to SBS16 reported in [73] using data from [62]. [4][52]. *p-value < 0.05 (Wilcoxon Signed-Rank Test), **p-value < 0.01.

Summary of Findings

In a small group of families, we identified a weak statistical association between the enrichment of mutation signature SBS16 in the germline and complex chromosomal aberrations in the veteran father. We also observed a significant positive trend between increased unstable aberration burden in veteran fathers—including complex aberrations—and higher frequencies of chromatid aberrations in their adult children within the high-SBS16 subgroup.

Although this observation could infer a relationship between cytogenetic markers of radiation exposure in the father (complex aberrations) and markers of effect (genomic instability) in their adult child, cautious interpretation is required. The many caveats (small subgroups, presence of controls in high-SBS16 subgroup, lack of radiation dosimetry), all downplay the confidence of this finding. Further research in larger cohorts with known radiation exposures is needed to explore these preliminary associations.

In the broader context of health concerns reported by nuclear test families, we find no statistically significant associations between any reported health-related variables and unstable aberration burden in either veteran fathers or their adult children.

Concluding Remarks

In conclusion, this study found no chromosomal evidence of past radiation exposure in most nuclear test veterans sampled, offering reassurance that their attendance at nuclear test sites was not associated with detectable levels of radiation exposure. A small number of veterans—categorised *a priori* as having a higher risk of exposure—did show increased average levels of complex chromosomal aberrations. This may suggest internal contamination from α-particle emitting radioactive fallout in a very small sub-set of veterans.

For adult children of nuclear test veterans, we found no evidence of constitutional chromosomal abnormalities or elevated genomic instability (when examined as a cohort), including among families that reported adverse health effects in one or more children or grandchildren. These findings should provide reassurance to concerned families, as no genetic effects or elevated chromosome aberration burdens in veteran fathers were observed that could be attributed to participation in nuclear tests.

A very small group of families did show a weak association between complex chromosomal aberrations in veteran fathers and an enrichment of germline mutations associated with mutation signature SBS16. This raises the possibility that SBS16 may reflect the molecular processing of radiation-induced DNA damage that, if corroborated, could serve as a candidate transgenerational biomarker of paternal radiation exposure. Indeed, the mutational processes thought to be associated with SBS16 is repair of bulky DNA lesions [72, 75]. However, this interpretation is cautioned given the small SBS16 subgroup also includes control families (N=8; 6 nuclear test and 2 control). Further, to date, the SBS16 mutational signature has only been reported in alcohol-associated liver cancers [76] with no reports of any relationship with radiation exposure. This SBS16 signature also appeared to be positively associated with chromosomal features of genomic instability in a very small group of adult children. This raises the possibility that mutation pattern SBS16, or an unidentified factor co-occurring in these families, is related in some way with transgenerational genomic instability. It is worth highlighting that four of the six nuclear test families within the high-SBS16 subgroup include veterans classified in the highest potential for radiation exposure category (rank 3), including two who had recorded doses of <1.5 mSv. Additionally, three of the eight nuclear test and control families in this subgroup self-reported a congenital condition within the family. While preliminary, these observations warrant further investigation in other human populations with known exposures to ionising radiation—especially those involving internal contamination with alpha-emitters—to both corroborate and to explore the biological significance, if any, of SBS16.

Overall, despite ongoing concerns raised by nuclear test families regarding adverse health outcomes, our study found no statistically significant relationship between paternal chromosomal aberration burden, germline mutation frequency, genomic instability, and self-reported family health issues. Therefore, the congenital and other health concerns reported in these families are not explained by our findings. This suggests that if there is any effect of historical radiation exposure in families recruited within the GCFT study, it is either too small to be detected by current methods or unrelated to the outcomes observed.

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