

**Comments on “New Zealand Nuclear Test Veterans’ Study
- A Cytogenetic Analysis” by RE Rowland et al
[A Report by the Institute of Molecular Biosciences, Massey University,
presented to the New Zealand Nuclear Test Veterans’ Association (2007)]**

Introduction

This report follows on from an earlier report by the same group of researchers that examined the frequency of sister chromatid exchange - a form of chromosome aberration - in blood samples taken from a group of 50 New Zealand nuclear test veterans and from a group of 50 matched controls (Rowland et al, 2005). The current report examines three other cytogenetic assays based on blood samples from the same groups of men. The following comments address specific aspects of the new report and their implications.

Selection of subjects

The description of methods used to identify the participants in this study is clear and the cohort selection criteria seem to be generally well-designed. It is clear from the full version of the report – which includes the appendices – that a very detailed questionnaire was used to obtain information from the study participants. In view of the important influence of age on some of the assays, it is good that the investigators took care to ensure that the controls were age-matched to the nuclear test veterans.

It should be noted the veterans who participated in this study were volunteers and may not be representative of New Zealand nuclear test veterans as a whole. In particular, some of the veterans may have decided to take part in the study because of concerns about their health, whereas the participation of controls is unlikely to have been influenced by health concerns. This may not pose a problem if health status *per se* is unrelated to the assay findings. However, treatment for, say, cancer could well influence the assay results. It is stated on page 9 of the report that none of the study subjects was currently receiving radiotherapy or chemotherapy, which might leave open the possibility that the assays could have been affected by prior treatments. On the other hand, it is stated on page 11 that the study excluded potential subjects “having received radiation treatment or chemotherapy”. The lead author has informed HPA that the latter statement is correct and that none of the study subjects had ever received radiotherapy or chemotherapy (RE Rowland, personal communication, 10 June 2007). Were it the case that the test veterans who took part in this study tended to have suffered greater ill-health than veterans who did not take part, this might have had some influence on the findings if the level of chromosome aberrations were related to illnesses other than cancer. However, the magnitude of any such effect is likely to be small.

Assays

The cell culture methods used are straightforward, except that the culture times are atypically long. The investigators discuss this point in the report. For stable damage, which is the main thrust of the work, this does not matter because the *in vitro* cell cycling is additional to probably a lot of *in vivo* divisions that will have occurred during the past 50 years. However, with hindsight, it is disappointing that the scoring was not confined to first *in vitro* division cells because of the unexpected level of unstable aberrations noted. This may have taken the investigators by surprise and, as they admit, the culture protocol renders the unstable damage data unusable. All that can be concluded is that they have probably underscored the true level of this damage and qualitatively it supports the idea that a substantial amount of the chromosomal damage may be more recent than the 1950s.

Statistical analysis

The G_2 and micronucleus assays show convincingly that the two cohorts have generally unremarkably DNA repair capacities. The few outliers by G_2 do not show up in the micronucleus test and their translocation results are not suggesting an unusual response. This is perhaps a point that the authors could have made too. Moreover, two standard deviations in the mean should contain 95% of values to indicate normal distribution. Among the 49 veterans' samples, there were two values outside this boundary for the G_2 assay, which is acceptable. Discarding these outliers would not significantly affect the mean or variance of the experimental data, or the overall conclusions of the study.

The main nub of the study is the comparison of the stable translocation frequencies in the veterans and controls, assessed using the mFISH assay. The authors carried out a two-sample Wilcoxon rank sum test in order to test for differences between numbers of stable translocations for the two cohorts. The Wilcoxon test was appropriate as the data are of unknown distribution. The p value of < 0.0001 for this test indicates highly statistically significant differences between the numbers of stable translocations for veterans and controls.

Using an alternative approach based on t-statistics, we have calculated that the probability of observing a mean number of stable translocations among the veterans of at least 29.38, given the control (population) mean of 10.05, is extremely small, approximately 2×10^{-10} . Thus there is a very small probability that the observed difference between the veteran and control data is due to chance alone.

Statistical power

Assuming that the true difference between the veterans and the controls in the mean number of stable translocations were equal to the value observed (ie. $29.38-10.05=19.33$) and that it had been planned to estimate this difference with 5% error (based on a 95% confidence interval), then there would have had to have been about 45 persons in the veterans' group. In this study, stable translocations were assayed in 49 men in each cohort and the statistical power (an indication of the precision of the results) was reasonably high (~ 74%). While these numbers are theoretically large enough for statistical resolution, the value of 49 cases is approaching the minimum value of about 45. It would have been desirable, therefore, to have had a larger study group. However, the total number of New Zealand veterans is relatively small (about 550) and the level of the response to the request to participate in the study - together with the strict selection criteria - limited the number of veterans in this investigation.

Potential for confounding

The authors controlled for a sensible range of confounders, the most important of which is age. Furthermore, the control frequency reported for translocations is in line with that found by other laboratories. The veterans tended to have smoked more than the controls, particularly in the past. However, the difference between the veterans and controls in the mean number of stable translocations persisted after adjusting for smoking status. Indeed, there was very little indication of a difference in the frequency of stable translocations between ever-smokers and never-smokers. This conforms to the current consensus view that any effect of smoking on this assay is likely to be small.

The authors decided to omit persons who had served in the New Zealand Navy from the control group, so as to avoid any impact of possible ship contamination. Consequently, whereas the veterans had been in the Navy, the controls were mostly ex-Army personnel or ex-policemen. This raises the question as to whether some facet of service in the Navy other than participation in the nuclear weapons tests might be correlated with the level of stable translocations observed many years later. There is no known factor that might confound the results in this way, although

the degree to which other occupational exposures might have a long-lasting influence on stable translocation levels is not fully understood.

Comparison of results across assays

Of the three assays studied here, only one of them (mFISH) showed a statistically significant difference between the veterans and the controls. However, this is the assay that is known to have the closest association with radiation exposure. Furthermore, whereas FISH is a biomarker of past exposure, the aim of the G2 and micronucleus assays was to examine DNA repair capacity. In these *in vitro* tests, blood sample aliquots were given a test dose and measured to see if there was any difference in response. The G2 and micronucleus assays were performed because of reports of people having been pre-adapted by previous radiation exposure and so repairing their DNA more efficiently when encountering a later dose. Consequently, the failure to detect a difference in the numbers of aberrations between the veteran and control groups using these two assays does not argue strongly against a radiation effect.

FISH assays were conducted for several UK nuclear test veterans during the 1990s and did not indicate differences between veterans with cataracts and those without cataracts (Phelps-Brown et al, 1997). However, the UK study was small and – during the past decade – there have been substantial developments in the methodology for FISH. In particular, whereas early applications of FISH involved painting only a few chromosomes, using mFISH, every chromosome in the genome is painted. In addition, the New Zealand veterans took part in only one test operation (Grapple), whereas the UK veterans participated in a wider range of tests.

Estimation of doses

The *in vitro* dose response curves, which were used to derive tentative dose estimates for the veterans are inadequate. The dicentric curve, done with 96-hour cultures is quite suspect. FPG staining is mentioned, but the authors seem to skirt around why this was done. It should be to guarantee that first division metaphases were scored, but at 96 hours there would be remarkably few of them. However, the authors do play down the value of this curve. The translocation curve ideally should have been done with the same mFISH method, culture time and so on as used for the study cohorts. However, the genome equivalence adjustment is a reasonable procedure, given that only three pairs of chromosomes were painted for the purposes of dose reconstruction.

If the cytogenetic damage is more recent than the 1950s, this could support the possible role of long-lived and long-retained radionuclides. However, this has to be judged against what is known about the deposition patterns following the detonations and the positions of the ships (said to have been up-wind).

Implications for health

The authors point out on page 6 of their report that this study does not address the health status of the test veterans. Follow-up studies in various countries of populations with raised levels of chromosome aberrations have shown increased risks of cancer (eg. Boffetta et al, 2007). However, chromosome aberrations do not appear to be markers of health damage *per se* but rather indicate past exposure to genotoxic agents.

If the estimates of radiation doses to the New Zealand veterans based on the *in vitro* dose response for translocations were correct, then it might be expected that an elevated cancer rate would have been detected in the epidemiological study of New Zealand test veterans (Pearce et al, 1990, 1997). As it turned out, this study did not find an increase in cancer overall, although there were indications of a raised leukaemia risk. However, the New Zealand cohort was small,

so limiting the precision of this study, although among the larger group of UK participants in Operation Grapple there were no indications of raised cancer risks (Muirhead et al, 2003). Furthermore, for the reasons stated earlier, estimates of doses based on combining the mFISH findings with the *in vitro* dose response are uncertain.

Press coverage of the New Zealand report has touched upon the implications for the health of the offspring of the veterans. Again, the presence of chromosome aberrations would not be an indicator *per se* of ill-health in offspring. Furthermore, whilst various epidemiological studies have shown raised risks of cancer in populations exposed to high or moderate radiation doses (UNSCEAR, 2000), this is very little evidence from human studies to suggest to raised risks of ill-health in offspring, although a small increase might be expected on the basis of radiation genetics (UNSCEAR, 2001). In particular, whereas studies of the survivors of the atomic bombings of Hiroshima and Nagasaki have shown raised levels of chromosome aberrations (Cologne et al, 1998) and increased risks of cancer risks (Preston et al, 2003), studies of their offspring have not demonstrated increases in ill-health (Izumi et al, 2003a,b).

Conclusions

We concur with the authors that the results from this study indicate a statistically significant threefold increase in stable translocations for veterans compared to controls and that it is possible to ascribe the increase in stable translocations to radiation exposure. However, the unstable aberrations data, which have only a qualitative value, leave open the question of whether all of the dose was delivered around the time of the nuclear weapons tests.

Possible future work

The following issues could be considered:

- (i) Further attempts could be made to obtain more information on possible levels of radiation exposure to participants in Operation Grapple, based on contemporary records.
- (ii) The mFISH technique could be applied to a larger group of nuclear test veterans, such as those in the UK. However, in order to confirm or deny the New Zealand results, it would be necessary to include a sizeable number of veterans from Operation Grapple – possibly about twice the number of New Zealand veterans - as well as considering participants in other operations. In addition, careful consideration would need to be given to the selection of subjects, given that many of the veterans are now in their 70s or 80s and that the opportunity to collect blood samples appropriate for mFISH analysis – together with questionnaire data – is likely to diminish as the veterans age further. Since such a study would take several years to undertake, preparatory work would need to start fairly soon in order for it to be at all feasible.

Radiation Protection Division

Health Protection Agency

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