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Elevated chromosome translocation frequencies in New Zealand nuclear test veterans

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Abstract. In 1957/58 the British Government conducted a series of nuclear tests in the mid-Pacific codenamed Operation Grapple, which involved several naval vessels from Britain and New Zealand. Two New Zealand frigates with 551 personnel onboard were stationed at various distances between 20 and 150 nautical miles from ground zero. In the present study we applied the cytomolecular technique mFISH (multicolour fluorescent in situ hybridisation) to investigate a potential link between chromosome abnormalities and possible past radiation exposure in New Zealand nuclear test veterans who participated in Operation Grapple. Compared to age matched controls, the veterans showed significantly higher ($P < 0.0001$) frequencies of chromosomal abnormalities (275 translocations and 12 dicentrics in 9,360 cells vs. 96 translocations and 1 dicentric in 9,548 cells in the controls), in addition to a significant excess of CCRs (complex chromosomal rearrangements) in the veterans. A Kolmogorov-Smirnoff test showed that the distributions of translocations for the two groups were significantly different.

Operation Grapple was the codename given to the British series of nuclear tests that were conducted at Christmas Island and Malden Island in the mid-Pacific in 1957/58. Among the naval vessels which took part in the operation were two New Zealand frigates with 551 personnel onboard that were stationed at various distances between 20 and 150 nautical miles from ground zero (Crawford, 1989). Although several studies have been conducted regarding the health of nuclear veterans from Britain, USA, Australia and New Zealand (Pearce, 1990; Pearce et al., 1990; Rabbitt Roff, 1999; Dalager et al., 2000; Muirhead et al., 2003), the small number of participants in the New Zealand group was always going to make epidemiological studies of this cohort difficult, as any radiation-induced cancers that might result would not easily be detectable against background incidences within the spectrum of different cancers that may arise spontaneously (McEwan, 1988). Nevertheless, some studies have found moderate increases in the incidences of haematological cancers in the New Zealand veterans, such as leukaemia (Pearce et al., 1990).

The present report describes excess chromosomal damage in the blood lymphocytes of 49 New Zealand military personnel (nuclear test veterans, mean age 65.9 years) compared to 50 controls matched in age (mean age 66.5 years) and regional origin. Multicolour-FISH (mFISH) analysis of

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all chromosomes using spectral karyotyping was performed to score chromosomal abnormalities after 72 h of blood lymphocyte culture. Each chromosome was painted in an individual colour, permitting structural aberrations to be identified throughout the genome in each individual metaphase. The introduction of mFISH into radiation cytogenetics has not only widened the spectrum of aberrations amenable to detection, but has also made it possible to refine the detection and definition of complex chromosomal rearrangements that were hitherto only possible with conventional staining.

Material and methods

Study design

Population and sampling procedure. Fifty male New Zealand naval nuclear test veterans and 50 male age-matched controls who had also undergone military or police training when they were younger, participated in the study. One veteran's lymphocytes failed to grow, reducing the size of this group to 49. All males were selected by the following procedure.

Veterans were initially sent a 'Preliminary Inclusion Criteria Questionnaire'. From information furnished, a potential participant pool was formed by excluding any respondents who failed to meet specific inclusion criteria. A final participant pool was formed by randomly selecting the specified number of participants from the potential participant pool database.

Age-matched control subjects were selected from a pool of volunteers according to strict criteria identical to those for the veterans, but with the essential difference that they did not participate in Operation Grapple. Ex-servicemen were selected as controls, most, as was intended, from the army. The main aim of this criterion was to control for the healthy soldier bias (Bross and Bross, 1987). Some ex-policemen were also chosen. Ex-naval servicemen were excluded as control subjects on the grounds of controversy as to whether the frigates involved were completely 'clean' upon returning to New Zealand and whether they may have subsequently been manned by other crews who theoretically may thus have been exposed to contamination. Ex-airforce personnel, except for ground crew, were also excluded, for reasons of possible increased past exposure to cosmic radiation. Vietnam veterans were also not included in either the control or veterans group in view of the risk that these people may have been adversely affected by exposure to defoliants (Rowland et al., 2007). To control for any environmental variation, selection of both veterans and controls was geographically stratified across the North Island of New Zealand. Other exclusion criteria are listed below.

The selected final participants completed an information sheet, consent form and detailed questionnaire that gathered further information relating to their medical history, occupational history and lifestyle history to refine the matching of the two groups. Following the return of the questionnaire, a face-to-face interview was arranged and conducted by a psychologist skilled in interviewing techniques. This allowed clarification of some questionnaire responses and, where necessary, further questioning to obtain more detailed information. A blood sample was collected at the time of the interview, or else arrangements were made to collect a sample from the participant at a later convenient date. The whole study was conducted following strict ethical guidelines as specified by the World Medical Association Declaration of Helsinki. Ethics approval to conduct the study was given by the Massey University Human Ethics Committee (PN Protocol 01/61).

Each blood sample was collected by an independent phlebotomist and coded with a number so that the researchers could eventually link a name with that code. This code, without the name, was written on the side of each blood tube and delivered to the Massey University Student Health Clinic in Palmerston North. Medical assistants at the clinic recoded each tube with a new number and kept a record linking the codes that were eventually revealed at the conclusion of the study. This ensured that no member of the research team could identify a veteran from a control. The blood samples were then collected from the clinic for genetic analysis. The study was thus conducted blind to rule out any bias during analysis. The codes were broken and veterans/controls identified only after all cytogenetic analyses were completed.

Exclusion Criteria. Participants were excluded if they 1) had served in a theatre of war or nuclear-related area, 2) were exposed, for a year or more, to toxic substances which included asbestos, tanilised timber, oil/petrol fumes, microwave radiation, road transport (dust and chemicals) and radiography work, 3) had received radiation treatment or chemotherapy, 4) were aged over 75, 5) had been air force aircrew, 6) were too ill to participate, 7) died subsequently before survey completion.

Cytogenetic procedures

Peripheral blood lymphocytes were cultured for 72 h, pretreated with 0.05% colchicine for 1 h, harvested and fixed, then sent in 2 ml Eppendorf tubes to Dr Ilse Chudoba at Metasystems GmbH, Germany, who performed the preparation of the probe mixture for all 46 chromosomes and in situ hybridisations (Chudoba et al., 1999). C-metaphases were automatically located using a metaphase finder Metafer, captured with an image analyzer, and stored on disc for analysis in our laboratory using the ISIS programme (Metasystems).

Scoring criteria. The scoring criteria of Whitehouse et al. (2005) were followed. Aberrations were classified according to the PAINT nomenclature of Tucker et al. (1995), and classification of complete exchange aberrations was performed according to S&S nomenclature (Savage and Simpson, 1994). Analysis was conducted on intact metaphases where all 46 painted chromosomes could be identified. Cells with 45 chromosomes which exhibited balanced translocations were also recorded. Translocations were recorded in stable cells only, including complete translocations, incomplete translocations and Robertsonian translocations. Translocations were not scored if they occurred in complex cells (defined as three or more breaks on two or more chromosomes) or in any unstable cell (dicentrics, acentrics or rings involving any chromosomes) as most were too complicated to score accurately. Reciprocal exchanges were designated as t(Ab) + t(Ba) and incomplete exchange as t(Ab) and a deleted chromosome. Each complete translocation was counted as one translocation. Robertsonian translocations were cytologically distinct from satellite fusions; in the latter the satellites are adjacent to each other but the chromosomes are not fused. Difficulty in identifying dicentrics and acentrics in several complex cells led us to score such aberrant chromosomes and fragments only in cells that were, according to the above definition, not complex.

Examples from nuclear test veterans are illustrated: Figure 1 is a normal c-metaphase spread, whereas Fig. 2a shows a complete translocation. Profiles of the translocated chromosomes identify an exchange between chromosomes 1 and 3 (Fig. 2b, c). Figure 3 is an example of an incomplete translocation where the long arm of chromosome 12 has transferred onto chromosome 1. Figure 4 is an example of an unstable cell showing several translocations and a dicentric chromosome involving chromosomes 4 and 8. Figure 5 shows the profile of a single chromosome with multiple translocations from a complex cell. Figure 6 is a very complex cell. Although insertions were observed in complex cells, none were observed in the stable cells scored in either the veterans or controls.

Because we were looking at possible long term exposure, we restricted the scoring to stable cells. To ascertain the frequency of stable aberrations many years after exposure, lymphocytes are normally cultured for 46–50 h. Our cultures were harvested after 72 h to accommodate a number of different assays (G2, SCE, COMET, MN), which failed to show differences of radiosensitivity or repair capacity between veterans and controls (data not shown). Some of the cells in our study may thus have gone through two or three cell cycles, which was deemed acceptable because previous studies have established that there is no decrease in translocation yield after three to four divisions (Guerrero-Carbajal et al., 1998). If, however, cells pass through more than one cell cycle, then clonality becomes an issue when scoring translocation frequencies. We were particularly alert to this possibility, but in all our observations of translocations in each individual, no clonal cells were detected.

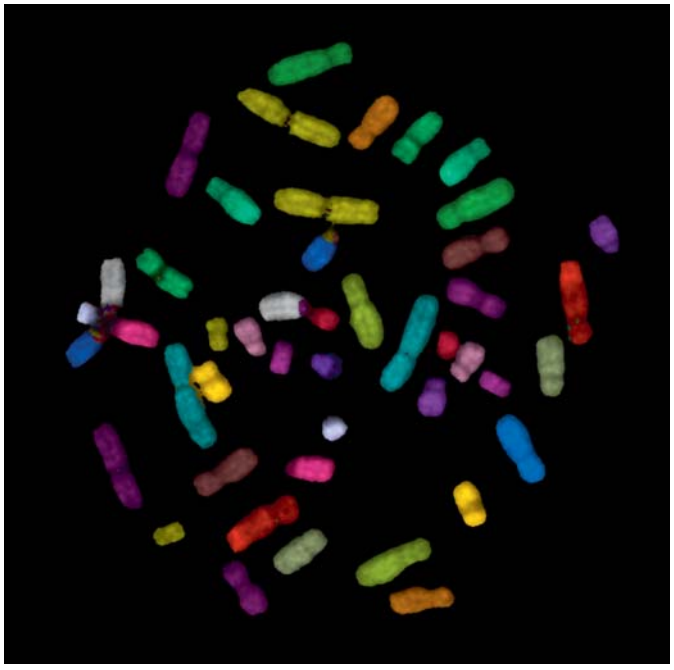

Fig. 1. C-metaphase spread of a normal karyotype from a dividing human peripheral blood lymphocyte, painted via mFISH.

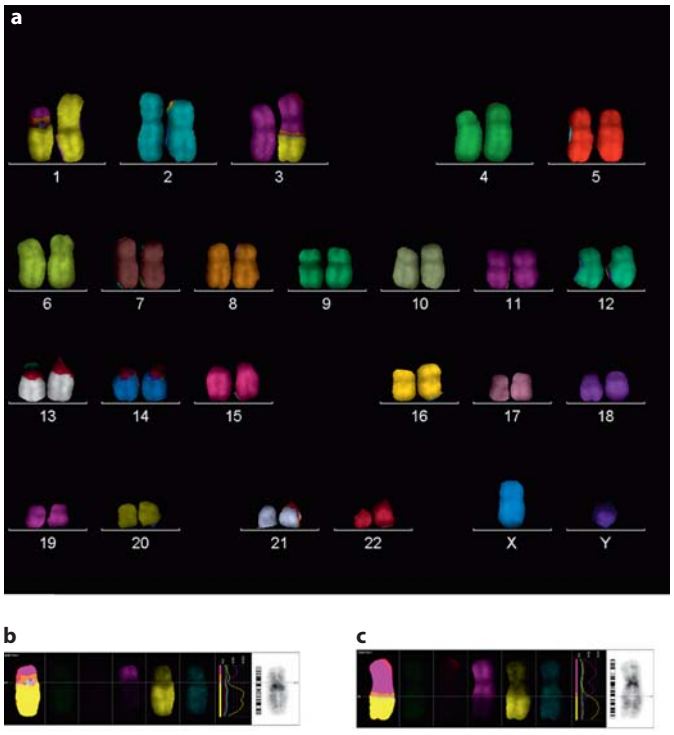

Fig. 2. (**a**) Karyotype of a dividing blood lymphocyte showing a reciprocal exchange between chromosomes 1 and 3. (**b**, **c**) Chromosome profiles of chromosomes 1 and 3.

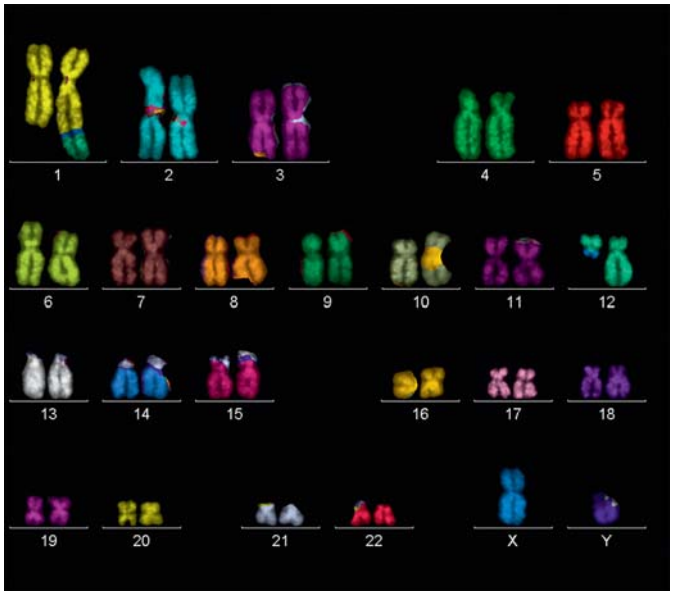

Fig. 3. Karyotype of a dividing blood lymphocyte showing the transfer of the long arm of chromosome 12 onto chromosome 1. What appears to be an insertion in chromosome 10 is a chromosomal overlap.

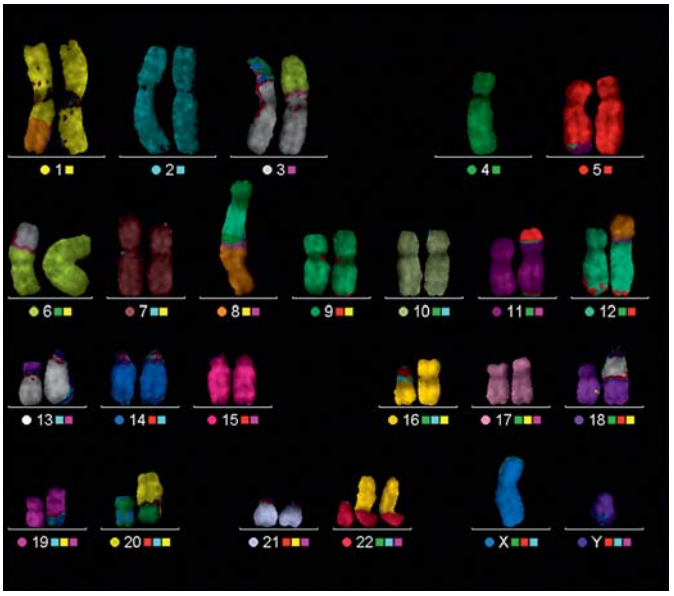

Fig. 4. Karyotype of a dividing blood lymphocyte showing multiple chromosome exchanges.

In vitro irradiation and lymphocyte culture. With informed consent, a blood sample from a healthy donor (60 years) was analyzed for chromosomal abnormalities after in vitro irradiation with 250 keV x-rays at 0, 0.2, 0.5, 0.75, 1, 2 and 4 Gy. Whole blood was added to the culture medium with 2% phytohaemagglutinin M (GIBCO/BRL, USA) for 72 h. Blood was incubated at 37°C for 2 h in the presence of colcemid (0.1 μg/ml) before harvesting. Slides were prepared after standard methanol/acetic acid (3/1, v/v) fixation and stored at –20°C until use (M'Kacher et al., 2007).

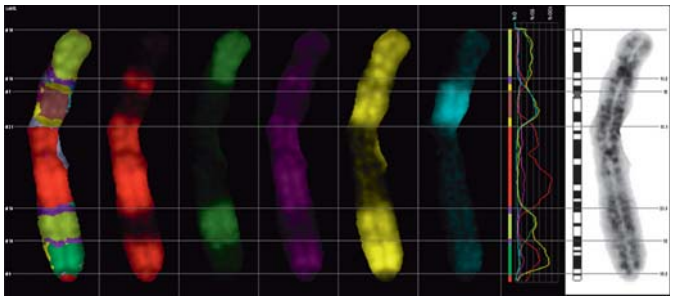

Fig. 5. Profile of a multitranslocated chromosome comprising material from chromosomes 5, 6, 7 and 9.

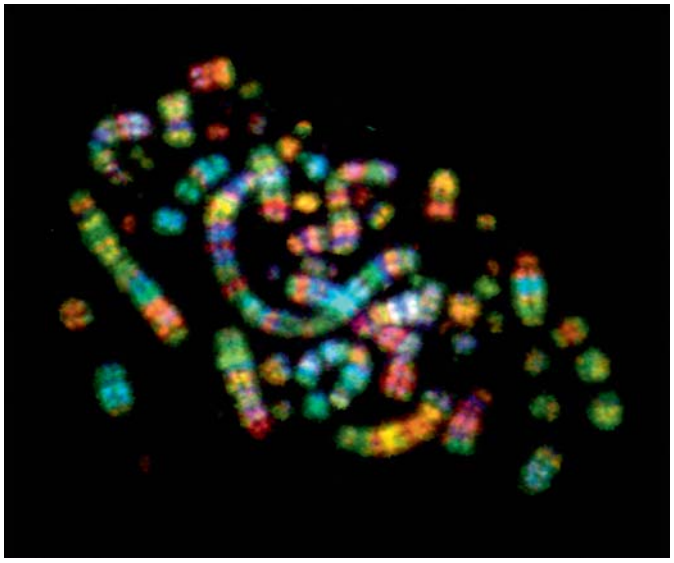

Fig. 6. Chromosomal spread of a dividing blood lymphocyte showing multiple complex exchanges.

Fluorescence in situ hybridisation (FISH) for dosimetric analysis. Slide pretreatment: slides were rinsed for 30 min at 37°C in 2× SSC/0.5% NP-40 and dehydrated for 2 min in three successive alcohol solutions (70, 85, and 100%). They were then denatured for 2 min in 70% formamide/2× SSC at 72°C and dehydrated.

Probe preparation: the probe was pre-warmed at 37°C for 5 min. An aliquot of 3 μl of the red total chromosome 1 DNA probe, 3 μl of the green total chromosome 4 DNA probe, 1.5 μl of the red and 1.5 μl of the green total chromosome 3 DNA probe (Appligene Oncor) was used for hybridisation.

Results and discussion

From a total of 9,360 cells scored in the veterans, 226 cells were observed with one or more translocations (Table 1). A total of 275 translocations were recorded, comprising 94 complete translocations, 136 incomplete translocations and 45 Robertsonian translocations. In the control group, 88 cells were observed with one or more translocations, from a total of 9,548 cells scored (Table 2). A total of 96 transloca-

Table 1. Raw data for translocation frequency in stable cells and dicentric/acentric frequency in the veterans group

Participant	Cells[a]	T[b]	Cells with T[c]	RT[d]	2-way[e]	1-way[f]	dic[g]	ace[h]
NZTV-001	14	1	1	0	0	1	1	0
NZTV-003	203	8	4	0	2	6	1	13
NZTV-010	200	3	3	0	0	3	0	0
NZTV-011	200	4	4	0	1	3	0	2
NZTV-017	202	5	5	0	1	4	0	1
NZTV-018	201	3	2	0	0	3	0	4
NZTV-019	201	4	3	0	0	4	0	4
NZTV-020	200	7	7	1	4	2	0	0
NZTV-021	154	6	5	2	1	3	2	0
NZTV-023	201	5	5	2	3	0	0	1
NZTV-024	201	7	6	1	1	5	0	4
NZTV-025	204	8	7	2	3	3	0	10
NZTV-026	201	13	9	2	1	10	0	2
NZTV-033	203	10	8	2	2	6	0	3
NZTV-034	202	4	3	2	0	2	0	0
NZTV-037	No available data							
NZTV-039	200	7	4	1	1	5	1	1
NZTV-041	204	10	6	2	3	5	1	4
NZTV-042	201	9	9	0	7	2	1	0
NZTV-043	201	9	5	0	4	5	0	3
NZTV-044	203	8	7	1	3	4	1	1
NZTV-045	205	5	5	1	2	2	0	0
NZTV-046	203	3	1	0	1	2	0	0
NZTV-047	106	2	2	1	1	0	0	0
NZTV-048	188	7	5	1	2	4	0	1
NZTV-049	202	11	5	1	2	8	0	0
NZTV-050	201	4	4	0	4	0	0	0
NZTV-051	203	3	3	0	2	1	0	0
NZTV-052	202	1	1	0	0	1	0	0
NZTV-053	92	0	0	0	0	0	0	2
NZTV-055	209	9	9	0	2	7	0	1
NZTV-056	217	3	3	0	0	3	0	0
NZTV-057	167	1	1	0	0	1	0	0
NZTV-058	145	0	0	0	0	0	0	1
NZTV-060	164	0	0	0	0	0	0	0
NZTV-061	220	2	2	0	2	0	0	1
NZTV-062	200	13	9	1	6	6	0	0
NZTV-064	206	6	4	0	6	0	1	4
NZTV-065	182	2	2	0	2	0	0	0
NZTV-066	202	12	10	0	8	4	0	1
NZTV-068	208	4	3	0	3	1	0	1
NZTV-069	204	8	8	3	2	3	1	1
NZTV-070	205	5	7	3	0	2	0	0
NZTV-072	205	7	4	2	1	4	1	0
NZTV-073	214	3	3	2	0	1	0	1
NZTV-074	205	9	8	4	3	2	1	1
NZTV-075	203	7	8	4	1	2	0	5
NZTV-076	206	7	7	2	3	2	0	0
NZTV-079	199	4	4	2	1	1	0	0
NZTV-097	201	6	5	0	3	3	0	4
	9,360	275	226	45	94	136	12	77

[a] Number of cells karyotyped.
[b] Total number of stable translocations (RT + 2-way + 1-way).
[c] Number of cells with translocation(s).
[d] RT = Robertsonian translocation.
[e] 2-way = complete translocation.
[f] 1-way = incomplete translocation.
[g] dic = dicentric.
[h] ace = acentric.

Table 2. Raw data for translocation frequency in stable cells and dicentric/acentric frequency in the control group using mFISH

Participant	Cells[a]	T[b]	Cells with T[c]	RT[d]	2-way[e]	1-way[f]	dic[g]	ace[h]
NZTV-002	86	0	0	0	0	0	0	2
NZTV-006	200	3	3	0	0	3	0	0
NZTV-012	203	2	2	0	2	0	0	1
NZTV-013	71	0	0	0	0	0	0	2
NZTV-014	150	1	1	0	0	1	0	0
NZTV-015	203	0	0	0	0	0	0	1
NZTV-016	201	1	1	0	0	1	0	1
NZTV-022	201	3	3	0	1	2	0	1
NZTV-027	201	2	1	0	1	1	0	1
NZTV-028	202	2	1	0	1	1	0	1
NZTV-029	203	0	0	0	0	0	0	2
NZTV-030	200	1	1	0	0	1	0	2
NZTV-031	202	2	2	0	1	1	0	1
NZTV-032	202	3	3	0	2	1	0	2
NZTV-035	202	0	0	0	0	0	0	0
NZTV-036	203	4	1	0	1	3	0	0
NZTV-040	202	4	3	0	2	2	0	4
NZTV-059	144	4	3	0	0	4	0	0
NZTV-063	200	4	4	2	0	2	0	0
NZTV-067	205	4	4	2	1	1	0	1
NZTV-071	202	2	2	0	2	0	0	1
NZTV-077	203	1	1	0	1	0	0	0
NZTV-078	203	0	0	0	0	0	0	2
NZTV-080	204	0	0	0	0	0	0	1
NZTV-081	203	1	1	0	1	0	1	0
NZTV-082	202	1	1	0	1	0	0	1
NZTV-083	203	2	2	0	1	1	0	0
NZTV-084	200	1	1	0	1	0	0	0
NZTV-085	203	3	3	0	3	0	0	1
NZTV-086	202	2	2	0	1	1	0	1
NZTV-087	201	0	0	0	0	0	0	1
NZTV-088	200	0	0	0	0	0	0	1
NZTV-089	201	2	2	0	1	1	0	1
NZTV-090	202	1	1	0	0	1	0	0
NZTV-091	203	0	0	0	0	0	0	0
NZTV-092	201	1	1	0	0	1	0	0
NZTV-093	106	0	0	0	0	0	0	0
NZTV-094	89	1	1	0	0	1	0	0
NZTV-095	203	1	1	0	1	0	0	4
NZTV-096	203	7	7	2	3	2	0	2
NZTV-098	200	1	1	0	1	0	0	1
NZTV-099	190	2	2	0	1	1	0	3
NZTV-100	201	6	6	0	4	2	0	0
NZTV-101	200	1	1	0	0	1	0	0
NZTV-102	203	3	3	0	3	0	0	1
NZTV-103	202	3	3	0	2	1	0	2
NZTV-104	229	2	2	0	1	1	0	2
NZTV-105	203	7	7	1	2	4	0	1
NZTV-106	202	2	2	0	0	2	0	0
NZTV-107	203	3	2	0	3	0	0	0
	9,548	96	88	7	45	44	1	48

[a] Number of cells karyotyped.
[b] Total number of stable translocations (RT + 2-way + 1-way).
[c] Number of cells with translocation(s).
[d] RT = Robertsonian translocation.
[e] 2-way = complete translocation.
[f] 1-way = incomplete translocation.
[g] dic = dicentric.
[h] ace = acentric.

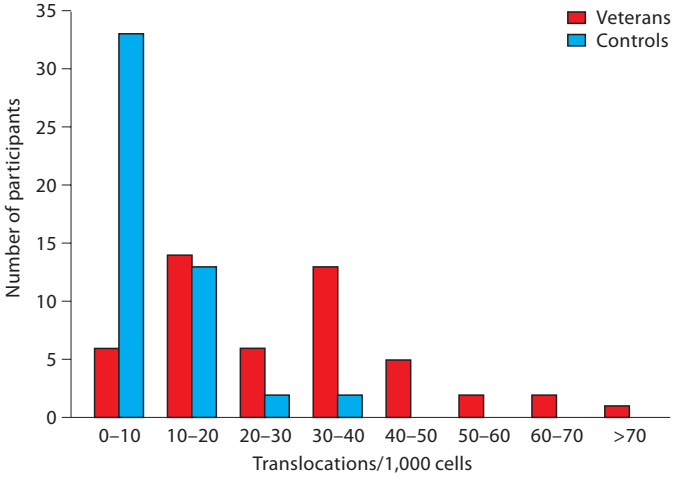

Fig. 7. Graph showing the distribution of total translocation frequencies (RT, complete and incomplete) in stable cells in the veterans and the controls as a function of the number of translocations per 1,000 cells.

Table 3. Data showing the mean translocation frequencies per 1,000 cells for stable cells in the veterans and the controls

Group	No	Mean	Sd	Sem	95% CI for mean	
					Lower bound	Upper bound
Veterans	49	29.38	17.52	2.50	24.08	34.15
Controls	50	10.05	8.86	1.25	7.29	12.32

No = Number of participants; Sd = standard deviation; Sem = standard error; CI = confidence interval.

tions were recorded, comprising 45 complete translocations, 44 incomplete translocations and seven Robertsonian translocations. A Wilcoxon two sample rank sum test revealed a highly significant increase in the number of translocations per 1,000 cells in the veterans (mean = 29.38) compared to the controls (mean = 10.05), $W = 385$, $P < 0.0001$ (Table 3).

The distribution of the total translocation frequencies expressed as the number of translocations per 1,000 cells in the veterans and the controls are different between the two groups (Fig. 7). The variance is greater in the veterans, with a range of 0–65 translocations per 1,000 cells compared to 0–35 translocations per 1,000 cells in the controls. The control group is heavily represented in the category of 0–10 translocations per 1,000 cells. After equal exposures and with equal numbers of evaluated cells per person – and accepting that multiple translocations per cell from densely ionizing radiation and different sensitivities of persons are being disregarded – the numbers of translocations should follow a Poisson distribution. On the other hand, unequal exposures, exposures from different radiation types or different contaminations are known to produce yields of aber-

Table 4. The mean total translocation frequencies per 1,000 cells between smokers and never-smokers in the veterans and the controls

Group	Smoking status	No	Mean	Sd	Sem	95% CI for mean	
						Lower bound	Upper bound
Veterans	Smoker	37	28.21	14.37	2.36	23.41	32.99
	Never-smoker	12	31.94	25.54	7.37	15.70	48.16
Controls	Smoker	33	9.20	8.29	1.44	6.25	12.13
	Never-smoker	17	11.00	10.03	2.43	5.84	16.15

No = Number of participants; Sd = standard deviation; Sem = standard error; CI = confidence interval.

rations that do not conform to a Poisson distribution (IAEA, 2001). A Kolmogorov-Smirnoff test of the unexposed controls in our study also showed good agreement with a Poisson distribution with a steep decline at higher translocation frequency. This, however, is not the case in the New Zealand veterans. Although the sample size in our study was not sufficient to state with certainty that the distribution of translocations in the veterans is not Poissonian, Fig. 7 clearly indicates a frequency distribution in this group that is multimodal in accordance with complex exposures. A Pearson scale factor was added to account for overdispersion as reported in retrospective biodosimetry of radiologic technologists (Bhatti et al., 2007), but this did not affect our analysis.

A further notable difference in our data is a higher ratio of incomplete to complete translocations in the veterans (136:94), whereas the controls showed a ratio close to 1:1 (44:45). Most in vitro studies on radiation-exposed peripheral blood lymphocytes after a uniform single exposure show a proportion of complete to incomplete translocations roughly equal to 2:1 (Lindholm et al., 2002; Braselmann et al., 2005; Roy et al., 2006). It is interesting to note that the minimal detectable size of translocated chromosome segments using mFISH is 11.1 Mb (Kodama et al., 1997), whereas human telomeres are only 5–15 kb long. Studies using PNA telomeric probes have established that the true percentage of incomplete exchange patterns is approximately 3–5% (Wu et al., 1998; Fomina et al., 2000, 2001). The majority of incomplete aberrations arising from terminal exchanges are in fact unresolvable using FISH (Boei et al., 1998; Fomina et al., 2001). Similarly, these authors note that in order to identify true complete translocations, it is advisable to use telomeric probes. We attribute the unexpected comparatively high incomplete translocation frequencies to deficiencies in resolution of the mFISH technique, especially in older men. Indeed, it is likely that we have underscored the number of complete translocations in our study and thus our results are conservative. Moreover, telomere alterations can be a source of chromosome instability and can result in complex chromosome rearrangements (M'Kacher et al., 2007). The introduction of PNA telomeric probes in future studies could clarify this issue.

The significantly higher translocation frequencies in the group of veterans compared to the controls suggests that this may be a consequence of their participation in Operation Grapple, i.e. the nuclear tests of the years 1957/58. However, since statistical association is not necessarily proof of a causal relation, possible confounders need to be considered.

As there has been close matching for age and lifestyle in the study design, these factors are not considered to be confounding variables in our study. In addition, there was no significant difference in present smoking level between the two groups, with nearly all participants currently being non-smokers. The higher rate of past smoking among the veterans as compared with the controls might be at least partially responsible for the higher translocation rates in the veterans. If this were so, then the translocation rates should be higher for the smokers in both the veteran and control groups, and, as Table 4 shows, this clearly is not the case. A between-groups analysis of variance with group as one factor and smoking as the other showed no effect of smoking, $F < 1$. In the veterans group, the mean number of translocations per 1,000 cells was in fact a little higher in the never-smokers (mean = 31.94, SD = 25.54) than in the smokers (mean = 28.21, SD = 14.37). Similarly for the controls; the never-smokers (mean = 11.00, SD = 10.03) had a slightly higher mean translocation rate than the smokers (mean = 9.20, SD = 8.29). Thus, smoking was not a factor influencing translocation frequency.

For reasons that have been stated, the control group was made up of military personnel who were not serving on naval vessels. Thus, there might, in principle, have been a confounding factor specific to service on naval vessels. Although one might consider chemicals from fresh paint or similar agents, it is difficult to conceive of such a factor causing the highly significant and lasting increase in translocation frequencies.

Whether radiation exposure during Operation Grapple or contaminations incurred by the naval personnel can have been the causative factor, is not easily answered. It will require careful reconsideration of the type and magnitude of potential exposures. Retrospective biodosimetry from translocations has been extensively used (IAEA, 2001; Edwards et al., 2005). After informed consent, in vitro exposures of a blood sample of a normal donor of age 60 were, therefore, performed for a rough retrospective biological

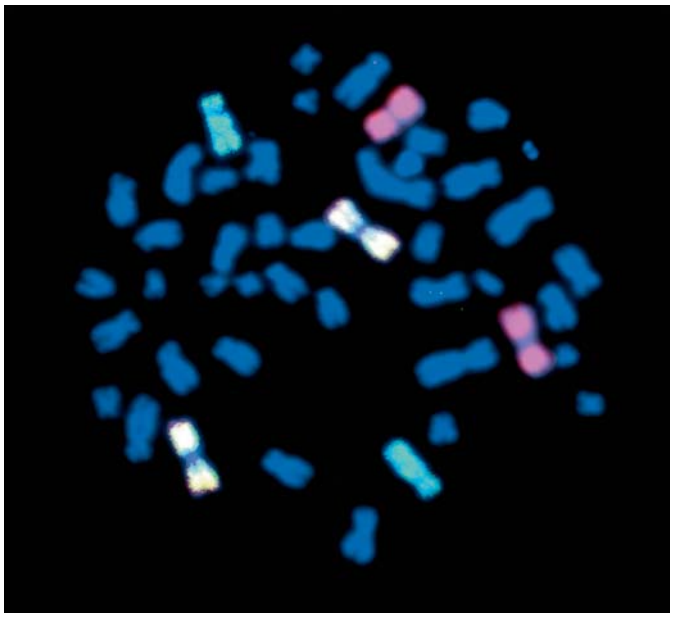

Fig. 8. Human karyotype from a dividing peripheral blood lymphocyte showing chromosomes 1, 3 and 4 painted for dosimetric analysis.

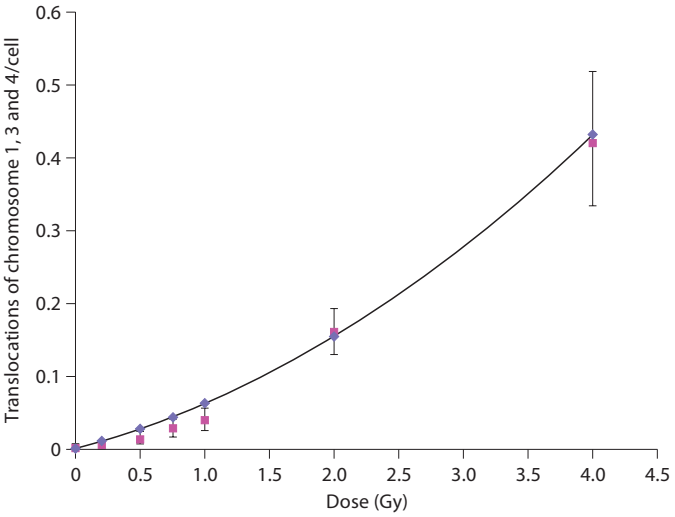

Fig. 9. Dose response curve derived from translocations scored after in vitro irradiation with x-ray in peripheral blood lymphocytes (pink dots) showing a linear-quadratic response with alpha and beta coefficients of 0.045 Gy^{-1} and 0.015 Gy^{-2}, respectively (purple dots). The 95% confidence interval is plotted. The coefficients for the response curve, extrapolated to the entire genome using Lucas' formula, are 0.117 Gy^{-1} and 0.039 Gy^{-2}, respectively.

dosimetry in order to indicate the magnitude of exposures, which might have been responsible for the observed translocation frequencies among the veterans.

Retrospective biological dosimetry, with overdispersion allowance, was attempted from the mFISH data of the veterans, by comparison to an extrapolated dose-response curve, obtained by Lucas' formula (Lucas et al., 1993) after scoring translocation frequencies observed in chromosomes 1, 3 and 4 in the exposed blood sample (Figs. 8, 9). This classical extrapolation was applied in our study since good correlation was observed between the number of translocations observed on chromosomes 1, 3 and 4 with the number of translocations observed in each participant involving all chromosomes (r = 0.7), and since the mFISH study excluded a clonal bias in the veterans (Pouzoulet et al., 2007). The values thus inferred are, of course, the x-ray doses equivalent to the potential doses of an uncertain radiation type and irradiation modality. Dose estimates ranged from 0 to 0.431 Gy in the veterans (mean = 0.170 Gy) and from 0 to 0.22 Gy in the controls (mean = 0.037 Gy). The difference between the mean doses was highly significant ($P < 0.0001$). Moreover, the dosimetric index was significantly higher than 0.2 Gy in 43% of veterans vs. 4% in the controls ($P < 10^{-6}$), (Fig. 10). While the biodosimetry just after single doses of x-rays can only give an approximate dosimetric index, this index is still a useful indicator that can be taken into consideration even after complex irradiations (Bhatti et al., 2007).

The comparatively higher frequency of dicentrics and acentrics scored in the veterans (12 dicentrics and 77 acentrics) compared to the controls (1 dicentric and 48 acentrics) parallels the results found in French Polynesians with thyroid cancer (Violot et al., 2005). The high frequency of dicentric chromosomes and acentric fragments observed in the veterans, many of them in very complex cells that could not be scored, is particularly salient. This finding is evocative of irradiation and further suggests that the veterans may have been contaminated and may have retained high-LET long half-life radionuclides in their bodies (Rowland et al.,

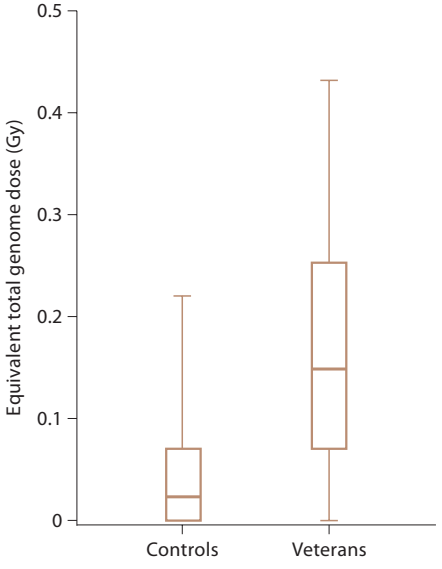

Fig. 10. Distributions of the mean absorbed dose (box) showing a marked difference between the veterans and controls. The middle line is the median and the lower and the upper limits are the minimum and maximum estimated doses.

2005). This is in line with the findings of other investigators who have reported that past radiation can leave a permanent signature in the genome several years after the event, as evident in plutonium workers exposed in 1949 (Hande et al., 2003), patients treated with x-irradiation for ankylosing spondylitis (Buckton, 1983), Hiroshima atomic bomb survivors (Kodama et al., 2001; Nakano et al., 2001), and more recently, Chernobyl cleanup workers (Lazutka, 1996; Slozina et al., 1997). Therefore, our results are not extraordinary, despite a gap of 50 years after the initial event.

A notable feature amongst the veterans in our study was the high number of cells with extraordinarily complex chromosomal rearrangements (CCRs) involving numerous translocations, apparently multicentric chromosomes, deletions, aneuploidy, centric and acentric fragments (Figs. 4, 5 and 6). Such chromosomally unstable cells were termed 'rogue' cells by Awa and Neel (1986). A random sample of ten veterans showed a total of 37 CCR cells, whereas the number of CCR cells observed in the entire control group amongst the thousands of cells observed, totaled less than ten. There is general agreement in the literature that there is no correlation between exposure to ionizing radiation per se and the occurrence of rogue cells, except possibly in one study on astronauts exposed to high-LET radiation (Mustonen et al., 1998). Nonetheless, the presence of CCRs in veterans may be viewed as an additional indicator of past radiation exposure. Lazutka (1996) reported that rogue cells are seen in patients after nuclear accidents concomitant with a stimulation of JC-virus antibodies. This is not surprising considering that the immune system is known to be compromised by exposure even to low levels of ionizing radiation (Godekmerdan et al., 2004; Kusunoki and Hayashi, 2008). The high rogue cell count we observed in the veterans could be interpreted as a signature of immunodeficiency, arising initially as a consequence of radiation exposure. Viral infection alone, on the other hand, may not be sufficient to explain the extent of chromosome damage observed in some cells. A controversial suggestion, despite the above consensus, is that the complexity of anomalies observed may be explained by exposure to a heterogeneity of past and possibly present irradiations (protraction and multiplicity of exposures, contaminations with long half-life radionuclides). A complete study of such CCRs for the assessment of their dosimetric value would be a monumental but useful task.

Conclusion

In summary, a sample group of New Zealand naval personnel who participated in Operation Grapple shows three times the frequency of total chromosome translocations than a group of closely matched controls. Our analysis of potential confounding factors leads us to the view that this highly elevated frequency is most likely attributable to radiation exposure. Further clarification might be attained by a similar study on British and Fijian participants in Operation Grapple.

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