Increased frequency of chromosome translocations in airline pilots with long-term flying experience

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ABSTRACT

Background: Chromosome translocations are an established biomarker of cumulative exposure to external ionising radiation. Airline pilots are exposed to cosmic ionising radiation, but few flight crew studies have examined translocations in relation to flight experience.

Methods: We determined the frequency of translocations in the peripheral blood lymphocytes of 83 airline pilots and 50 comparison subjects (mean age 47 and 46 years, respectively). Translocations were scored in an average of 1039 cell equivalents (CE) per subject using fluorescence in situ hybridisation (FISH) whole chromosome painting and expressed per 100 CE. Negative binomial regression models were used to assess the relationship between translocation frequency and exposure status and flight years, adjusting for age, diagnostic *x* ray procedures, and military flying.

Results: There was no significant difference in the adjusted mean translocation frequency of pilots and comparison subjects (0.37 (SE 0.04) vs 0.38 (SE 0.06) translocations/100 CE, respectively). However, among pilots, the adjusted translocation frequency was significantly associated with flight years ($p = 0.01$) with rate ratios of 1.06 (95% CI 1.01 to 1.11) and 1.81 (95% CI 1.16 to 2.82) for a 1- and 10-year incremental increase in flight years, respectively. The adjusted rate ratio for pilots in the highest compared to the lowest quartile of flight years was 2.59 (95% CI 1.26 to 5.33).

Conclusions: Our data suggests that pilots with long-term flying experience may be exposed to biologically significant doses of ionising radiation. Epidemiological studies with longer follow-up of larger cohorts of pilots with a wide range of radiation exposure levels are needed to clarify the relationship between cosmic radiation exposure and cancer risk.

Airline pilots are exposed to ionising radiation of galactic and solar origin[1] and are considered to be a radiation-exposed occupational group in many countries.[2] The cosmic radiation field at aircraft altitudes consists mainly of secondary neutrons and gamma radiation, with some protons, alpha particles and heavy nuclei.[3 4] It has been estimated that approximately 60% of the cosmic radiation exposure of pilots is from high-linear energy transfer (LET) radiation, particularly neutrons which are generated due to interactions with the atmosphere.[5 6] Pilots are also potentially exposed to other chemical and physical agents in the working environment, such as jet fuel and engine emissions as well as electromagnetic fields from cockpit instruments.[7]

Since the 1990s, reports of elevated risks of cancers of various sites among pilots have raised concerns about the potential adverse health effects of workplace exposures, particularly cosmic ionising radiation. Thus far, the epidemiological findings on cancer risks among pilots are inconsistent, and except for significant increases in the risk of malignant melanoma and non-melanoma skin cancers, no clear patterns for cancers of other sites have been observed.[8 9] Furthermore, of the cancers that have been reported among pilots, only acute myeloid leukaemia and occasionally colon and bladder cancers were associated with radiation.[10] The inconsistent findings have been attributed to epidemiological study limitations, including low statistical power to detect a small radiation effect, difficulty in identifying an appropriate comparison population, the accuracy of estimated exposure to cosmic radiation, and the lack of data on potential confounding factors.[7] Therefore, further studies that address these issues and that provide some insight into the mechanisms are needed to clarify the relationship between cosmic radiation exposure and cancer risk.

Ionising radiation is an efficient inducer of chromosome aberrations[11] which have also been shown to be associated with cancer risk.[12] Previous cytogenetic studies of flight crews have mainly focused on unstable chromosome aberrations such as dicentrics and rings with reports of significantly higher mean frequencies in flight crew members compared to non-flight personnel,[13–17] with some matched in age[14 15] or health and social status.[14] In contrast, no increased frequencies of dicentrics and rings were found in cabin attendants when compared to two groups of age-matched ground personnel and administrative workers.[18] The mean frequency of the overall group of chromosome aberrations (predominantly gaps and strand breaks) was reported to be higher in flight engineers than in ground personnel with similar distributions in age, smoking and alcohol consumption, but the difference was not statistically significant.[19] Unstable chromosome aberrations, however, are rapidly eliminated from circulation through mitotic death and are therefore suitable only for assessing recent radiation exposures.[20]

Translocations, the most stable form of structural chromosome aberrations, have been used to assess cumulative exposure to chronic and low-dose ionising radiation in several occupational settings.[21–27] Translocations can be quantified in peripheral blood lymphocytes using the established cytogenetic method of fluorescence in situ hybridisation (FISH) with whole chromosome paints. In the current study, we assessed the relationship between translocation frequency and (a) exposure

status (airline pilots vs comparison subjects) and (b) duration of flight experience (as a proxy measure of dose) among pilots, adjusted for potential confounders.

METHODS

Study population

Male pilots of a major US airline were recruited from a city with a large number of international pilots. A list of full-time pilots was obtained from the union representing the pilots. A comparison group consisting of currently employed male university faculty members was recruited from the same city. They were identified from the database of a commercial company using information on graduation year, positions held and type of work performed. University faculty members were selected because they were comparable to pilots in several factors, including demographics, socioeconomic status and gender distribution.

All potential study subjects were sent an introductory letter and were then screened in a telephone interview by trained interviewers to determine study eligibility based on the following criteria: age 35–56 years; a never smoker (defined as a person who smoked a lifetime total of <100 cigarettes) or a smoker with limited smoking history (defined as a smoker who had not smoked in the last 10 years or who was currently smoking <10 cigarettes per day); no personal history of cancer except for non-melanoma skin cancer; no history of chemotherapy or radiotherapy (except routine diagnostic *x* ray procedures); and no family history of chromosomal instability disorders such as ataxia telangiectasia, Fanconi's anaemia, Bloom's syndrome or xeroderma pigmentosum. In addition to the above eligibility criteria, pilots were also selected based on duration of employment and years of flying international flights. Our objective was to recruit a larger group of pilots than comparison subjects to allow some variation in exposures in pilots that would permit internal comparisons in the analyses. For the comparison subjects, to ensure similarity to pilots except for occupational cosmic radiation exposure, eligibility also included the following criteria: no history of excessive past air travel (defined as no more than an average of one international flight/year and one domestic flight/month); no history of major illnesses such as heart disease and diabetes (which are disqualifying health conditions for pilots); and not being employed in a clinical or laboratory area that involves exposure to radiation or chemotherapeutic drugs. The study was approved by the Human Subjects Review Boards of the National Institute for Occupational Safety and Health and the National Cancer Institute, and all subjects provided written informed consent.

Data collection and covariates

Enrolment of pilots and comparison subjects was conducted between December 2001 and September 2002 at a medical clinic of an international airport and a university health services clinic, respectively. Each subject provided a venipuncture blood sample which was coded and shipped overnight to the cytogenetics laboratory. All study subjects completed a detailed self-administered questionnaire that was verified for completeness by interviewers at the time of blood collection with telephone follow-up if necessary. Information collected included the following: demographics, occupational history (including exposures to substances at work and occupational physical activity level), medical history (including medication use, vaccinations and family history of cancer), height, weight, smoking and alcohol consumption history, recreational physical activity, and cumulative segments of personal air travel (a segment defined as a single flight between any two cities without layovers/stopovers or intermediate flights).

The questionnaire also elicited information on routine personal diagnostic *x* ray procedures including the number and calendar year of each type of examination. For each subject, a cumulative red bone marrow (RBM) dose was estimated using the mid-point RBM dose values in cGy of specific procedures obtained from a comprehensive list of examination types.[28] Since the RBM dose values did not differ substantially over time from 1960 to 2001, total doses for each procedure type for each subject were calculated by multiplying the number of examinations by the corresponding mid-point dose.[21] Total doses were summed over all procedures to obtain an estimate of the cumulative personal diagnostic RBM *x* ray dose. We refer to this estimate as a dose score, rather than dose per se, because of the potential for uncertainties in recall of various procedures and uncertainties with the dose estimates for each procedure.

FISH assay for chromosome translocations

The analysis of chromosome translocations by FISH was conducted by laboratory personnel without knowledge of the exposure category of the study subjects. Cell cultures were initiated on blood samples within 24 h of blood draw and were processed in accordance with routine cytogenetic methods.[29] The slide preparation, staining and cell scoring were performed using standardised FISH protocols.[29 30]

Briefly, whole blood (0.8 ml) was added to 10 ml of RPMI 1640 medium supplemented with 15% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 1% sodium heparin and 2 mM L-glutamine and stimulated with 2% phytohaemagglutinin. Cultures were incubated at 37°C in a 5% CO_2 environment for 52 h, the last 4 h with 0.1 μg/ml colcemid to block mitosis.[29] The cells were harvested by swelling in a hypotonic solution (0.075 M KCl) following centrifugation and fixed a minimum of three times with 3.1 (v/v) methanol:-glacial acetic acid. The fixed cells were then dropped onto slides, taking care to minimise the presence of cytoplasm. After drying in air, the slides were stored at −20°C in sealed plastic bags in the presence of a desiccant and N_2 atmosphere until needed for hybridisation.

Chromosomes 1, 2 and 4 were painted red and chromosomes 3, 5 and 6 were painted simultaneously in green by using probes purchased from Cytocell Technologies (Cambridge, UK). The slides were then counterstained with 4′,6-diamidino-2-phenylindole (DAPI), mounted in an antifade solution[31] and stored at 4°C until they were scored. This combination of paints detects 56% of all the chromosome exchanges.[30 32] All cells were visualised using a fluorescence microscope with a triple band pass filter, which allowed simultaneous viewing of the labelled and DAPI counterstained chromosomes. Only well-spread metaphase cells that met established criteria[29 30] were scored. Digital photographs of all abnormal cells were obtained.

All translocations were initially classified according to the Protocol for Aberration Identification and Nomenclature Terminology (PAINT).[33] Subsequently all translocations, whether reciprocal or non-reciprocal, were counted as single translocations based on the premise that most non-reciprocal translocations are reciprocal at the molecular level for low doses or low dose rates. For each subject, the electronic images of all cells with translocations were evaluated to determine if any of the cells were clones. A clone is defined as a minimum of three cells with the same translocation and is considered as a single

translocation event.[34] Only one out of 133 subjects had clones. A total of approximately 1800 metaphase cells were evaluated per subject, and this was equivalent to $1800 \times 0.56 = 1000$ metaphase cells as if the full genome had been scored (defined as cell equivalents (CE)). The translocations in all cells of each subject were counted and totalled as the translocation frequency. In order to permit comparisons among subjects, the translocation frequency was converted to the full genome level, that is, expressed per 100 CE per subject.

Statistical analyses

All analyses were performed using SAS software v 9.0 (SAS Institute, Cary, NC), and a p value <0.05 (two-sided) was considered statistically significant. Means, standard deviations and standard errors were computed for translocation frequency by exposure status and by categories of covariates within each group, and among the pilots, by quartiles of duration of flight experience in years (hereafter referred to as flight years). For each pilot, flight years were computed from the commercial airline flight history reported in the questionnaire.

The unit of statistical analysis was the subject and not the cells. Negative binomial regression models were used to assess the relationship between translocation frequency (as the dependent variable) and (a) flight years (as a continuous variable) for all subjects (coded 0 for comparison subjects) as well as for pilots only, (b) exposure status (coded 1 for pilots and 0 for comparison subjects), and (c) quartiles of flight years (as a categorical variable) among pilots. Negative binomial regression was selected because it provides an efficient approach for the control of over-dispersion of count data which can result in increased unexplained variance and biased standard errors for the parameter estimates.[35] Rate ratios with the Wald 95% confidence intervals (95% CI) relating translocation frequency and flight years or exposure status were estimated by exponentiating the corresponding regression coefficient. The p values for the likelihood ratio χ^2 statistic were also computed since it is preferable for small sample sizes. Because age was correlated with the number of flight years among pilots ($r = 0.72$), the regression analyses were also performed stratified by 5-year age intervals (37–42, 43–48 and 49–55 years). The test for trend for the rate ratios across the quartiles of flight years among pilots was performed by treating the medians of each category as a continuous variable in the regression model. For ease of interpretation and comparison with other studies, the adjusted mean translocation frequency by exposure status and among the pilots, by quartiles of flight years was also computed with a linear model using analysis of covariance to adjust for potential confounders.

Potential confounders included in the multivariate negative binomial regression and the analysis of covariance models were those whose inclusion in the model resulted in a change in the association between exposure status or flight years and translocation frequency, that is, a change in the regression coefficient of at least 10%. The covariates included in the final models (as categorical variables) were: age at blood draw (41–45, 46–50 or >50 vs ⩽40 years as referent); cumulative RBM *x* ray dose score (0.5–1.9 or ⩾2.0 vs <0.5 as referent); and military flying (yes vs no). Cigarette smoking, exposures to substances at work, cumulative personal air travel, and other self-reported non-occupational and lifestyle characteristics did not materially alter the results and were not included as covariates in the final models. In addition, since the translocation frequency did not differ significantly between the never and ever smokers in both study groups, and similar results were obtained when we excluded a current smoker with 1.75 pack-years (packs of cigarettes smoked/day multiplied by years of smoking) in the comparison group, all subjects were included in the final analyses.

Table 1 Selected characteristics of pilots and comparison subjects

Characteristic	Pilots (n = 83)	Comparison group (n = 50)
Age at blood draw in years	46.7 (5.3), 37–55*	45.8 (5.0), 36–56
Body mass index in kg/m^2	26.5 (2.9), 20.2–35.1	26.7 (3.3), 20.4–39.0
Cigarette smoking		
Never	67 (80.7%)†	43 (86.0%)
Ever	16 (19.3%)	7 (14.0%)‡
Pack-years§	8.0 (9.7), 0.1–36	2.8 (3.6), 0.2–9
Drink alcohol		
No	4 (4.8%)	5 (10.0%)
Yes	79 (95.2%)	45 (90.0%)
Amount in g/day	11.4 (7.4), 1.3–34.9	14.6 (24.3), 0.8–162.7
Recreational activities past year		
Vigorous activities¶ (no. of months)	10.1 (3.4), 0–12	7.4 (5.2), 0–12
Family history of cancer (no. first degree relatives)		
None	39 (50.0%)	24 (50.0%)
One	35 (44.9%)	21 (43.8%)
Two or more	4 (5.1%)	3 (6.2%)
Unknown	5	2
Cumulative red bone marrow *x* ray dose score		
<0.5	33 (39.8%)	27 (54.0%)
0.5–1.9	39 (47.0%)	16 (32.0%)
⩾2.0	11 (13.3%)	7 (14.0%)

*Mean (SD), range; †n (%); ‡includes one current smoker with 1.75 pack-years; §packs of cigarettes smoked/day multiplied by years of smoking; ¶vigorous activities include sports, exercise class, swimming, heavy labour or any physical exertion that involves sweating.

RESULTS

Selected demographic and lifestyle characteristics of the study groups are shown in table 1. All pilots (n = 83) and comparison subjects (n = 50) were white males and were comparable with respect to age, mean body mass index, cigarette smoking and alcohol consumption. Pilots engaged in vigorous physical activity during more months of the year than the comparison subjects. The distribution of self-reported history of cancer among first degree relatives was similar for pilots and comparison subjects (table 1). Both groups also have similar distributions in self-reported medication use, vaccinations in the past year, years living with cigarette smokers in the home, physical activity level at work, and number of months engaged in moderate physical activity during leisure time in the past year (data not shown). Pilots had a higher cumulative RBM *x* ray dose score than the comparison subjects (40% vs 54%, respectively for a score of <0.5 but 60% vs 46%, respectively for a score of ⩾0.5) (table 1). Fifty eight pilots (70%) reported serving in the military, whereas none of the comparison subjects had been in the military, and pilots had significantly more cumulative personal air travel than the comparison subjects (mean = 39 vs 22 flight segments, respectively, p = 0.01) (data not shown). Pilots reported an average of a total of 18.1 (range 1–37) flight years with an average of 5 years of international flying and 621 block hours (defined as the time interval between leaving the departure gate to arrival at the destination gate) per year (data not shown).

Table 2 Mean translocation frequency/100 cell equivalents by categories of covariates among pilots and comparison subjects

	Translocation frequency/100 cell equivalents					
	Pilots (n = 83)			Comparison group (n = 50)		
Characteristic	n (%)	Mean (SD)	p Value*	n (%)	Mean (SD)	p Value
Age at blood draw						
⩽40	13 (15.7%)	0.27 (0.19)	0.003	9 (18.0%)	0.17 (0.13)	0.02†
41–45	25 (30.1%)	0.29 (0.29)		16 (32.0%)	0.35 (0.38)	
46–50	16 (19.3%)	0.48 (0.36)		14 (28.0%)	0.40 (0.36)	
>50	29 (34.9%)	0.49 (0.36)		11 (22.0%)	0.28 (0.28)	
Smoking status						
Never	67 (80.7%)	0.37 (0.32)	0.26	43 (86.0%)	0.31 (0.33)	0.79
Ever	16 (19.3%)	0.48 (0.38)		7 (14.0%)‡	0.34 (0.29)	
Pack-years§						
0	67 (80.7%)	0.37 (0.32)	0.58	43 (86.0%)	0.31 (0.33)	0.84
⩽1	5 (6.0%)	0.53 (0.43)		4 (8.0%)	0.37 (0.35)	
>1	11 (13.3%)	0.45 (0.37)		3 (6.0%)	0.30 (0.26)	
Cumulative red bone marrow *x* ray dose score						
<0.5	33 (39.8%)	0.25 (0.20)	0.02	27 (54.0%)	0.31 (0.33)	0.45
0.5–1.9	39 (47.0%)	0.48 (0.33)		16 (32.0%)	0.32 (0.38)	
⩾2.0	11 (13.3%)	0.52 (0.49)		7 (14.0%)	0.31 (0.13)	
Military flying						
No	25 (30.1%)	0.31 (0.26)	0.11	50 (100%)	0.32 (0.32)	–
Yes	58 (69.9%)	0.43 (0.36)		0	–	–

*p Value (likelihood ratio χ^2 statistic) from separate univariate negative binomial regression models treating smoking status (ever vs never smokers) and military flying (yes vs no) as categorical variables, and age, pack-years and cumulative red bone marrow *x* ray dose score as continuous variables (p value for the linear term unless otherwise noted); †p value for the quadratic term. p = 0.38 for the linear term only; ‡includes one current smoker with 1.75 pack-years; §pack-years coded as 0 for never smokers. SD, standard deviation.

The mean translocation frequencies per 100 CE with respect to categories of covariates are shown in table 2. Among the pilots, there was a significant increase in the mean translocation frequency with increasing age (p = 0.003) and the cumulative RBM *x* ray dose score (p = 0.02). Among the comparison subjects, translocation frequency was found to have a non-linear and significant quadratic relationship with age (p = 0.38 for the linear term only and p = 0.02 for the quadratic term in a quadratic model), but was not associated with other variables. Translocation frequency was not associated with cigarette smoking (smoking status or pack-years) (table 2), other variables from table 1 (data not shown) or self-reported exposures to pesticides, petroleum products, solvents or dyes at work in both groups (data not shown).

Table 3 presents the results of the negative binomial regression model relating translocation frequency with flight years (as a continuous variable and coded 0 for comparison subjects). Among all subjects (model 1), translocation frequency was significantly associated with flight years univariately (p = 0.01 from the likelihood ratio χ^2 statistic, and the Wald 95% CI barely excludes 1.00); however, this was no longer significant after adjusting for age and other potential confounders (p = 0.34). Among the overall group of pilots (model 2), there was a significant association between translocation frequency and flight years, both univariately (p = 0.003) and multivariately (p = 0.01). The multivariate-adjusted rate ratio for a 1- and 10-year incremental increase in flight years was 1.06 (95% CI 1.01 to 1.11) and 1.81 (95% CI 1.16 to 2.82), respectively. When stratified by age groups, the multivariate-adjusted rate ratio for a 1-year incremental increase in flight years was 1.06 (95% CI 0.94 to 1.18), 1.05 (95% CI 0.96 to 1.15) and 1.08 (95% CI 1.03 to 1.13) for those aged 37–42, 43–48 and 49–55 years, respectively.

The mean translocation frequency was higher in pilots than in the comparison subjects (0.39 vs 0.32 translocations/100 CE), but was similar in both groups after adjusting for age and other potential confounders (0.37 vs 0.38 translocations/100 CE) (table 4). The results of the separate negative binomial regression models relating translocation frequency with exposure status and flight years (as a categorical variable) among pilots are also shown in table 4. Translocation frequency was not significantly associated with exposure status: the rate ratio for pilots versus comparison subjects was 1.25 (95% CI 0.92 to 1.70) univariately and 0.98 (95% CI 0.65 to 1.48) multivariately (model 1). Because age was non-linear in the comparison group, an interaction term between age group and exposure status was also tested in the regression model, but it was not significant either univariately (p = 0.31) or multivariately (p = 0.47) (data not shown). When pilots were categorised by quartiles of flight years, a general pattern of an increase in the mean translocation frequency with an increase in flight years was observed both before and after adjusting for potential confounders. There was a significant trend in the rate ratios across the categories of flight years, both univariately (p = 0.003) and multivariately (p = 0.007) (model 2). However, only the rate ratio for pilots in the highest compared to those in the lowest quartile of flight years was significant: 2.23 (95% CI 1.42 to 3.52) univariately and 2.59 (95% CI 1.26 to 5.33) multivariately.

DISCUSSION

We quantified translocations using FISH whole chromosome painting in a group of 83 male airline pilots and an external

Table 3 Association between translocation frequency/100 cell equivalents and flight years*

	Rate ratios† (Wald 95% CI)	p Value‡
Model 1 (pilots and comparison subjects)		
Flight years		
1-year increment		
Univariate	1.02 (1.00 to 1.03)	0.01
Multivariate§	1.01 (0.99 to 1.03)	0.34
Model 2 (pilots only)		
Flight years		
Overall		
1-year increment		
Univariate	1.04 (1.02 to 1.07)	0.003
Multivariate	1.06 (1.01 to 1.11)	0.01
10-year increment		
Univariate	1.54 (1.16 to 2.02)	0.003
Multivariate	1.81 (1.16 to 2.82)	0.01
Stratified by age intervals¶		
1-year increment		
Univariate		
37–42	1.02 (0.94 to 1.10)	0.59
43–48	1.00 (0.93 to 1.07)	0.90
49–55	1.07 (1.02 to 1.12)	0.005
Multivariate		
37–42	1.06 (0.94 to 1.18)	0.32
43–48	1.05 (0.96 to 1.15)	0.32
49–55	1.08 (1.03 to 1.13)	0.002

*Modelled as a continuous variable (coded as 0 for comparison subjects); †derived from the exponentiation of the corresponding negative binomial regression coefficient; ‡for the likelihood ratio χ^2 statistic; §adjusted for age (41–45, 46–50 or >50 vs ⩽40 years), cumulative red bone marrow *x* ray dose score (0.5–1.9 or ⩾2.0 vs <0.5) and military flying (yes vs no) as categorical variables; ¶5-year age intervals: 37–42 (n = 20); 43–48 (n = 28); 49–55 (n = 35).

comparison group of 50 male university faculty members. The mean translocation frequency of the pilots was found to be about 25% higher than that of the comparison group, but this increase was no longer observed after adjusting for age, cumulative RBM *x* ray dose score from personal diagnostic procedures, and military flying. However, among the pilots, the adjusted translocation frequency was found to be significantly associated with flight years. Adjustment for cigarette smoking, exposures to other substances at work, cumulative personal air travel, and other non-occupational or lifestyle characteristics did not materially alter the results.

Two prior studies have examined translocations among flight crew members using FISH whole chromosome painting. In the first study, Cavallo *et al*[36] compared chromosome aberrations between 48 pilots and flight technicians (mean age 54 years) and 48 ground personnel (mean age 53 years). Mean translocation frequencies for the exposed and comparison group were not reported. Using logistic regression, the study estimated a relative risk of 5.1 (95% CI 1.5 to 17.3) for translocations after adjusting for age, smoking and medication use. There was also an increase in the adjusted relative risk for translocations in the first three flight-hour categories (<11 350, 11 350–15 000, 15 000–17 000), but in the fourth category (>17 000) the risk decreased and was not significantly higher than that of the ground personnel. In the second study comprised of 11 non-smoking pilots and eight male professionals with a difference in age (mean 52 and 47 years, respectively; range 40–60), Nicholas *et al*[37] observed a significant difference in the mean translocation frequency between the two groups (0.0031 and 0.0010 translocations/CE, respectively, p = 0.03); however, the data were not adjusted for age or other potential confounders. No significant association was observed between the translocation frequency and a cumulative dose estimated using the reported flight history of pilots.

The current study has several strengths compared to the previous studies. It is the largest cytogenetic study to date of a homogenous group of commercial airline pilots. Airline pilots represent a highly select occupational group with health, occupational, non-occupational and lifestyle characteristics that may differ from the general population, in part due to their extensive medical evaluations at the time of employment and close medical surveillance throughout their career.[7] In this study, the comparison group of university faculty members was carefully selected to be comparable to the pilots in socio-economic status and other non-occupational and lifestyle characteristics, as confirmed by the questionnaire data. Similar characteristics include physical activity level at work, body mass index, medication use and alcohol consumption. In contrast, pilots had more frequent personal diagnostic *x* ray procedures than the comparison subjects, which may reflect the more frequent medical examinations they undergo to maintain their flying status. Therefore, as was done in previous studies of occupational groups with low chronic radiation exposure,[21] we have adjusted for this additional source of ionising radiation.

It is difficult to directly compare our findings on group differences in translocation frequency with those of earlier studies due to their incomplete reporting of results and the methodological differences. All the flight crew members included in the Cavallo *et al*[36] study were reported to have at least 18 years of flight experience compared to approximately 50% of the pilots in our study due to our selection of pilots to represent a wider range of flight experience. Other possible explanations for the conflicting findings include the difference in age distributions between the flight crew members and the comparison group, the use of different statistical analysis methods and/or adjustment for different potential confounders. In particular, the lack of adjustment for age can confound the results in important ways since age has been shown to account for as much as 70% of the variability in the frequency of translocations in subjects with no radiation exposure.[29]

An additional strength of our study compared to previous studies is the methodology that was used to measure translocations. Detection of translocations is dependent on the number of chromosomes painted and the number of colours used.[38] The percentage of all chromosome exchanges that can be detected in our study was 56% compared to the studies of Cavallo *et al*[36] and Nicholas *et al*,[37] which was 34%.[30 32] The sensitivity of detection of translocations is also dependent on the number of cells examined.[30 38] We evaluated an average of 1039 CE per subject compared to Cavallo *et al*[36] who evaluated an average of 100 CE per subject.

Translocation frequencies are known to increase with age. Among our pilots, age was also associated with flight years and cumulative exposure from diagnostic *x* rays. Although our results have been adjusted for age, cumulative exposure from diagnostic *x* rays and military flying, we cannot completely rule out that residual confounding could partially explain the relationship between flight years and translocation frequency among the pilots. However, when we further analysed our data within three age strata, the translocation frequency in the highest age category for our pilots (49–55 years) after adjusting for cumulative exposure from diagnostic *x* rays and military flying, was found to be significantly associated with flight years with a rate ratio that was comparable to those of the lower age

Table 4 Mean translocation frequency/100 cell equivalents and rate ratios by exposure status and flight years

		Univariate		Multivariate*	
	n (%)	Mean (SE)	Rate ratios† (Wald 95% CI)	Mean (SE)‡	Rate ratios (Wald 95% CI)
Model 1					
Exposure status					
Comparison group	50 (37.6%)	0.32 (0.05)	1.00	0.38 (0.06)	1.00
Pilots	83 (62.4%)	0.39 (0.04)	1.25 (0.92 to 1.70)	0.37 (0.04)	0.98 (0.65 to 1.48)
Model 2 (pilots only)					
Flight years§					
<13.17	21 (25.3%)	0.27 (0.05)	1.00	0.23 (0.10)	1.00
13.17–17.49	20 (24.1%)	0.42 (0.08)	1.50 (0.93 to 2.41)	0.38 (0.08)	1.39 (0.87 to 2.23)
17.50–23.24	21 (25.3%)	0.27 (0.06)	1.01 (0.62 to 1.65)	0.30 (0.07)	1.06 (0.57 to 1.97)
⩾23.25	21 (25.3%)	0.61 (0.08)	2.23 (1.42 to 3.52)	0.66 (0.09)	2.59 (1.26 to 5.33)
p Trend¶			0.003		0.007

*Adjusted for age (41–45, 46–50 or >50 vs ⩽40 years), cumulative red bone marrow *x* ray dose score (0.5–1.9 or ⩾2.0 vs <0.5) and military flying (yes vs no) as categorical variables; †derived from the exponentiation of the corresponding negative binomial regression coefficient; ‡derived from a linear model using analysis of covariance; SE involved pooling of unequal variance; §categorised by quartile cut points based on the flight year distribution of pilots; ¶derived from negative binomial regression model treating the medians of quartiles of flight years as a continuous variable.
SE, standard error.

group (37–42 years) as well as the overall group of pilots. The consistency of this result across age groups strengthens our confidence that the increased frequency of translocations of pilots was related to flight experience rather than residual confounding by non-occupational exposures that increase with age.

In this study, the adjusted translocation frequency of pilots was found to be significantly associated with flight years with an increase of 6% and 81% for a 1- and 10-year incremental increase in flight years, respectively. When pilots were categorised by quartiles of flight years, there was a significant trend of an increase in the adjusted rate ratios across the categories; however, only the rate ratio for the highest compared to the lowest quartile of flight years was substantially and significantly elevated. The exposure index used in this study, duration of flight experience, is an imprecise measure of the cumulative cosmic radiation dose because level of exposure depends on the routes flown as well as the number of flights per year. Nonetheless, this exposure metric is of interest because it is more easily obtained for record-based studies than route and flight frequency information that is needed to estimate the radiation dose. Duration of flight experience is also of interest because it is commonly used as an exposure metric in epidemiological studies of cancer risk among pilots and other flight crews. Moreover, in a validation study among pilots,[39] duration of employment was found to be highly correlated with total flight hours ($r = 0.84$) and with the cumulative dose ($r = 0.82$) estimated using job history data and CARI, which is a computer program that was developed by the Federal Aviation Administration to estimate doses received by individuals flying between airport locations.[40] This suggests that it is unlikely that our results based on flight years are biased appreciably by exposure misclassification. Computation of individual cumulative cosmic radiation doses from the detailed flight data obtained from our study pilots and the airline using CARI is currently underway. This will permit further analyses based on cosmic radiation dose estimates which will take into account the changes in altitude and latitude, and the stage of the 11-year solar cycle at the time of flight. This approach is expected to provide a more precise evaluation of the translocation frequency–cumulative cosmic radiation exposure relationship than the exposure surrogate of flight years or hours.

In summary, in this largest cytogenetic study of airline pilots utilising FISH whole chromosome painting to date, the adjusted translocation frequency was not significantly different from that of the comparison group. However, using internal comparison among pilots, the adjusted translocation frequency was found to be significantly associated with flight years. Translocations, the most stable form of chromosome aberrations, are an established biomarker of cumulative exposure to ionising radiation, which is an established human carcinogen. Our data suggest that pilots with long-term flying experience may be exposed to biologically significant doses of ionising radiation. Although the results of epidemiological studies on cancer risk among pilots have been inconsistent, many of these studies involved relatively short follow-up periods of relatively young cohorts and did not permit the evaluation of radiation-associated cancers. Longer follow-up of larger cohorts of pilots with a wide range of radiation exposure levels will be needed to clarify the relationship between cosmic radiation exposure and cancer risk.

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Ethics approval: The study was approved by the Human Subjects Review Boards of the National Institute for Occupational Safety and Health and the National Cancer Institute.

Main messages

- Airline pilots are exposed to cosmic ionising radiation which is an established human carcinogen.
- Chromosome translocations are an established biomarker of cumulative exposure to external ionising radiation.
- In this largest cytogenetic study of airline pilots utilising FISH whole chromosome painting to date, the translocation frequency of pilots was not significantly different from that of a comparable group of university faculty members, after adjusting for age and other potential confounders.
- When comparisons were made among pilots, the adjusted translocation frequency increased significantly with increasing flight years, suggesting that pilots with long-term flying experience may be exposed to biologically significant doses of ionising radiation.

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