



OPEN Suppressed DNA repair capacity in flight attendants after air travel

Sneh M. Toprani^{1,2}, Irina Mordukhovich^{2,3}, Eileen McNeely^{2,3} & Zachary D. Nagel^{1,2}✉

Elevated cancer risk and compromised reproductive health have been well documented in flight attendants (FA), but the etiology remains unknown. Many studies using cell and animal models suggest that air travel related exposures might plausibly explain the adverse health outcomes observed in flight crew, but our understanding of the underlying biological mechanisms is incomplete. During air travel, FA are constantly exposed to complex mixtures of mutagens in the flight cabin that may contribute to genomic instability by inducing DNA damage and interfering with DNA repair. Defects in DNA repair capacity (DRC) have been associated with risk of cancer and other diseases. To explore our hypothesis that alterations in DNA damage and repair in FA are related to flight travel, we conducted a pilot study of FA's DNA damage and assess global DNA repair efficiency pre and post flight. We collected venous blood samples from nine FA before and after flight. Differential blood cell counts were carried out to assess immune responses and functional assays were performed to assess the DNA damage response. The CometChip assay was employed to quantify baseline DNA damage and repair kinetics for DNA damage induced by X-rays. Fluorescence multiplex based host cell reactivation (FM-HCR) assays were utilized to assess DRC in five major DNA repair pathways. Our findings revealed a significant increase in lymphocyte counts as well as diminished repair of ionizing radiation induced DNA damage and excision of 8oxoG:C lesions in after flight samples. Our results illustrate the potential for using biological samples to identify molecular mechanisms that may implicate impaired genomic stability and altered immune responses in the etiology of excess cancer in FAs.

Keywords DNA damage, DNA repair, Blood count, Flight attendant, Cosmic ionizing radiation, Genomic integrity

Abbreviations

FA	Flight attendants
DRC	DNA repair capacity
FM-HCR	Fluorescence multiplex based host cell reactivation assays
BMI	Body mass index
PBMCs	Peripheral blood mononuclear cells
SSBs	Single strand breaks
DSBs	Double strand breaks
BER	Base excision repair
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining
HR	Homologous recombination
MMR	Mismatch repair
BF	Before flight
AF	After flight
CIR	Cosmic ionizing radiation
IR	Ionizing radiation

Flight crew (which include flight attendants, commercial pilots, and cabin crew) represent an understudied group of workers¹. Flight attendants (FA) are reportedly exposed to toxins in cabin air² and radiological exposures from cosmic radiation^{3,4}. They also experience many physical, biological, and psychosocial stressors, including stressful interactions with passengers and quick turnaround times between flights^{5–7}. Epidemiological

¹John B. Little Center of Radiation Sciences, Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA. ²Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA. ³SHINE, Human Flourishing Program Institute for Quantitative Science, Harvard University, Cambridge, MA, USA. ✉email: znagel@hsph.harvard.edu

studies have revealed multiple adverse health outcomes that are more common among FA when compared to the general population⁴, including breast and skin cancer as well as non-Hodgkin's lymphoma^{8–10}. However, the mechanism underlying this elevated cancer risk in FA remains unexplained.

Environmental and occupational exposures to DNA damaging agents can cause cell death, transcriptional stress, replication stress, senescence, and genomic instability^{11–13}, a key driver of carcinogenesis¹⁴. Occupational exposures can also promote DNA damage by triggering inflammatory responses that produce endogenous reactive oxygen species^{15–17}. Several DNA repair mechanisms operate in human cells to guard the genome against DNA damage caused by these exposures^{18–20}, and thus play a key role in protecting against cancer. Some of the major DNA repair mechanisms that eliminate DNA lesions are base excision repair (BER) for single strand breaks (SSBs)/abasic site/base damages²¹; nucleotide excision repair (NER) for bulky DNA adducts²²; non-homologous end joining (NHEJ) and homologous recombination (HR) for double strand breaks (DSBs)^{23,24}; and mismatch repair (MMR) for mismatched bases²⁵. Defects in DNA repair have been associated with increased risk of several diseases, particularly cancer^{20,26}. Chronic exposure to a complex mixture of stressors can cause an increase in allostatic load that can include long-lasting alterations in the efficiency of DNA repair^{20,27}. We hypothesized that altered DNA repair and immune responses could potentially explain excess cancer risk in FA³.

To address this question, we examined the effect of air travel on DNA damage and repair responses in FA. Blood samples were collected from FA before and after the flight travel and analyzed for DNA damage and alterations in DNA repair. Further, we implemented our well-established functional high throughput, Fluorescence multiplex based host cell reactivation (FM-HCR) assays^{12,28–31} to measure DNA repair capacity (DRC) for five major DNA repair pathways (NHEJ, BER, NER, MMR, and HR). Our assays included reporter plasmids with ionizing radiation-induced damage and site-specific lesions such as uracil, hypoxanthine, 8oxoG:C, and A:8oxoG. We carried out an unprecedented analysis of DNA repair using host cell reactivation technology in resting lymphocytes obtained from the FA blood samples. The data from this pilot study provide a basis for future, larger scale exploration in flight crew that could inform policies aimed at protecting the safety of flight crew and passengers, particularly pregnant women and frequent fliers who may be at higher risk than the general population.

Results

To investigate the impact of in-flight exposures on markers of genomic integrity in FA, we assessed several biological endpoints including differential blood cell counts, DNA damage levels, DNA repair kinetics, and global DNA repair capacity (DRC) analysis before flight (BF) and after flight (AF) travel in nine FA (Fig. 1a). The results of each analysis are summarized below:

Demographics, lifestyle and flight related parameters of study participants

Many factors including age, sex, smoking, drinking habits, exercise, sleep pattern, disease, or mental health may affect DNA damage and repair responses³² and were thus recorded through approved questionnaires (Supplementary doc. 1, Supplementary Fig. 1, Supplementary Tables 1 and 2) from the participating FA. Out of 9 FA, 3 were male and 6 were female (Supplementary Fig. 1a). All were of white ethnicity and were never smokers. Five were overweight based on body to mass index (BMI) calculated as the ratio of weight (in kg) and the square of the height of the participant (in m) (Supplementary Fig. 1b). In our study, the FAs age ranged from 24 to 64 years, with an employment duration designated as a FA position ranging from 2 to 41 years (Supplementary Fig. 1c). Age and the employment duration as a FA are associated with disease risk and chronic mutagenic exposures^{1,7,33}, and can provide an estimate of exposure to cosmic ionizing radiation (CIR), solar particle emissions, and other agents that increase the risk of cancer and other diseases^{4,34,35}. Details regarding sleep (Supplementary Fig. 1d), exercise and intake of medication (Supplementary Fig. 1e) and alcohol consumption (Supplementary Fig. 1f) were obtained. While our study was too small to adjust for lifestyle and demographic variables, our within-subjects study design allows for a straightforward comparison of short-term biological effects of air travel.

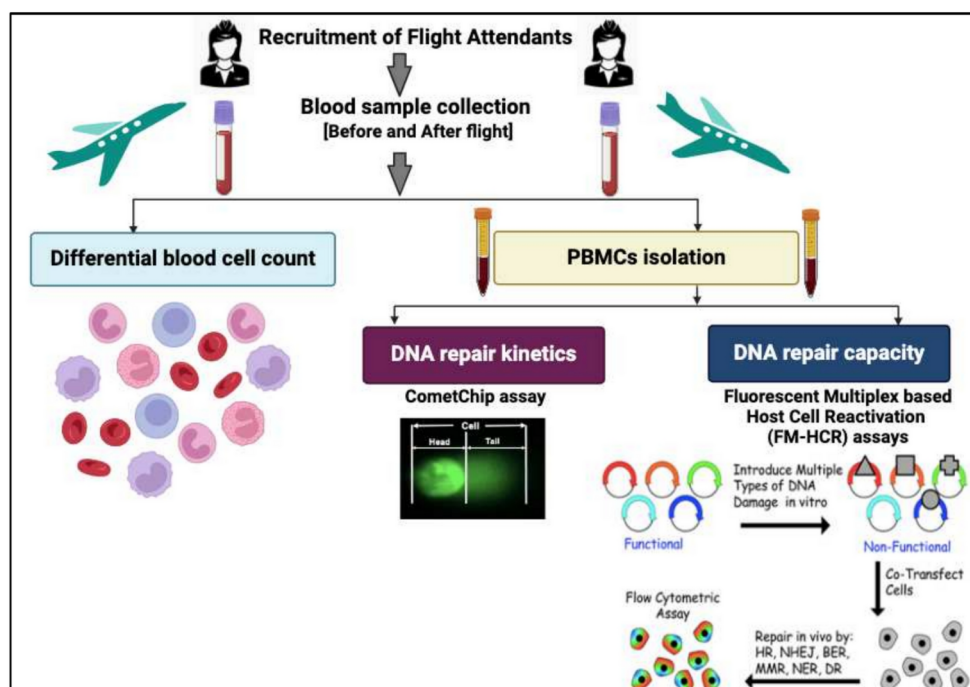
Changes in differential blood cell counts after flight travel

To assess the impact of flight travel on the immune system, we conducted differential blood cell counts. Blood samples for before flight (BF) measurements were collected 5–12 h prior to departure from Boston Logan International airport, Massachusetts, USA. After-flight (AF) samples were collected within 1.5 h of the landing to the same return airport (Fig. 1b). The duration between the departure from Boston and return varied from 27 h to 10 days, depending on the participating FA's flight schedule (Fig. 1b). Additionally, the interval since the last flight before the pre-flight blood collection ranged from 2 to 7 days (Fig. 1b). For some FA, we experienced challenges in drawing blood post-flight due to dehydration, which is common^{36,37}. Overall, there was no significant difference in the blood cell count landscape [white blood cells (WBC), red blood cells (RBC), neutrophils, monocytes, eosinophils, basophils, platelets, and hemoglobin (Hb)] between the BF and AF samples, except for Lymphocytes (Fig. 2a–c). We observed a significant increase in lymphocyte counts (adjusted $p=0.02$) (Fig. 3) in AF samples, potentially indicating an inflammatory response towards flight travel related exposures^{38,39}. Two FA showed notably higher basophil counts (Supplementary Fig. 2). Among the remaining FA, we observed a significant increasing trend in basophil counts (adjusted $p=0.03$) in AF samples (Supplementary Fig. 2), but when all data are included the trend does not reach significance due to the high variability arising from the two individuals with large increase in basophil counts.

Heterogeneity in DNA repair kinetics observed among individuals in after flight samples

Basal DNA damage and repair kinetics were measured using the alkaline CometChip assay in Peripheral blood mononuclear cells (PBMCs) isolated from nine FA blood samples. The percentage of DNA in the comet tail

a



b

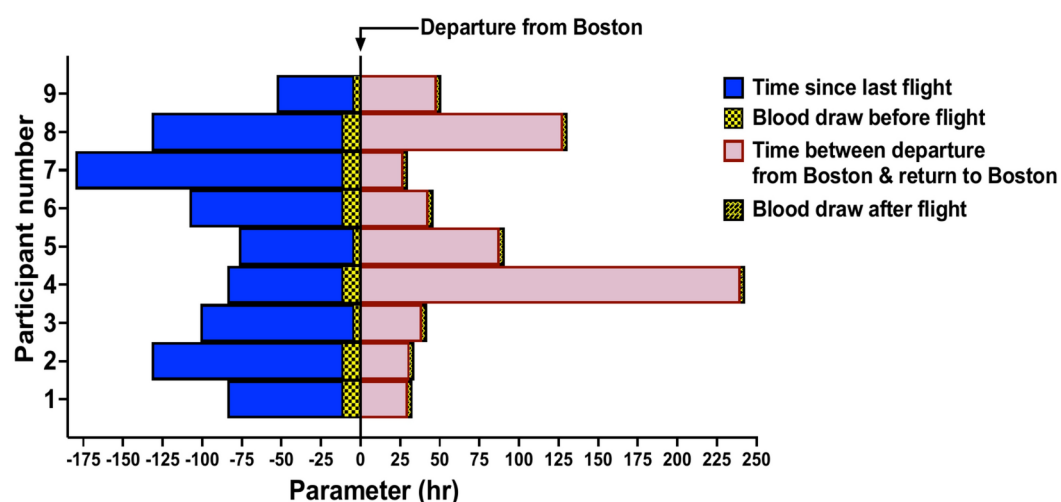


Fig. 1. Methodology implemented in this study. **(a)** Venous blood samples were collected from FA before and after flight travel. Differential blood cell counts were recorded. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation. The CometChip assay was used for quantitation of DNA damage & repair kinetics. Fluorescence Multiplex based Host Cell Reactivation (FM-HCR) assays were used for measuring DNA repair capacity. **(b)** Samples were collected from 9 FA (participant number indicated on the y-axis) immediately before departure and shortly after return from flight travel. The graph represents time in hours since the last flight (blue); the time between blood sample collection and flight departure (yellow and black checker pattern); and the time between departure from Boston to return to Boston airport (pink). After flight blood samples were collected immediately upon landing for nine FA (yellow and black closed crossed pattern, y-axis).

region (% Tail DNA) was used to estimate DNA damage levels. No statistically significant difference in the average basal DNA damage was found between samples taken BF (13.84 ± 9.70) and AF (11.17 ± 5.56) (Fig. 4a, b). Isolated PBMCs were irradiated at 4.0 Gy (X-rays, dose rate = 1.0 Gy/min) and IR-induced genomic DNA damage was measured at 0-, 5-, 15-, 30- and 60-min post irradiation to assess whether flight travel affects DNA repair kinetics in response to exposure to a secondary DNA damaging agent (Fig. 4c). The AF samples showed a subtle increase in genomic DNA damage at 5 min post irradiation, increasing the time (half time, $t_{1/2}$) to repair 50% of the initially induced radiation damage compared to BF samples. This increase in DNA damage suggests DNA repair dependent induction of SSBs intermediates, which might arise from BER processing oxidative DNA

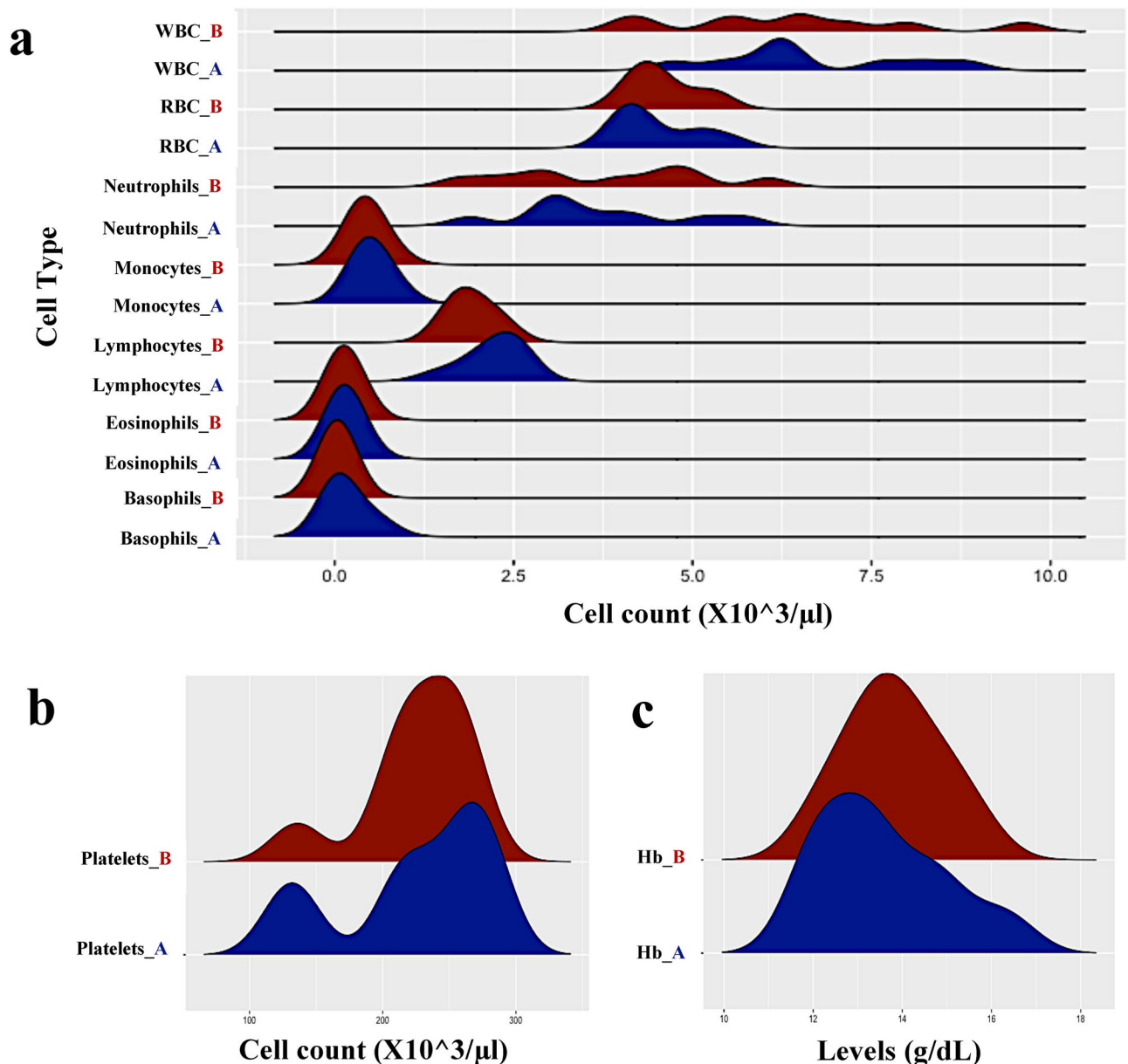


Fig. 2. Differential blood cell counts of FA before and after flight travel. Joyplot represents blood cell type counts ($10^3/\mu\text{L}$) obtained from before flight (maroon plot, Cell_Type_B) and after flight (blue plot, Cell_Type_A) for (a) White blood cell (WBC), red blood cell (RBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils, (b) platelets and (c) hemoglobin (Hb) levels (g/dL).

damage (Supplementary Fig. 3). Since this uptick in DNA damage was accompanied by a lower level of DNA damage at t_0 , it may reflect less efficient initiation of BER by DNA glycosylases (Supplementary Fig. 3). On an average, there was a statistically insignificant trend (adjusted $p=0.78$) towards slower repair capacity in AF samples ($K=0.033$, $t_{1/2}=25.59$ min) versus BF samples ($K=0.047$, $t_{1/2}=14.68$ min) (Fig. 4c). Most of the IR-induced genomic DNA damage was repaired within 60 min (Fig. 4c). Five participants exhibited slower repair kinetics in their AF samples (Supplementary Fig. 4), though these within-individual changes were generally smaller than inter-individual differences in repair capacity. Since some of the heterogeneity in $t_{1/2}$ observed in AF samples would be masked upon averaging, we categorized the FA repair kinetics data into two groups (Supplementary Fig. 5). Group 1 consisted of five FA whose AF samples showed slower repair kinetics ($K=0.012$, $t_{1/2}=65.40$ min) compared to their BF samples ($K=0.039$, $t_{1/2}=17.49$ min). Group 2 included four FA whose AF samples showed more rapid kinetics ($K=0.13$, $t_{1/2}=7.02$ min) than their BF samples ($K=0.053$, $t_{1/2}=13.02$ min). Overall, no significant difference in repair kinetics were observed among (Group 1: adjusted $p=0.44$; Group 2: adjusted $p=0.18$) and between (adjusted $p=0.45$) the two groups. Furthermore, classification as Group 1 or Group 2 was not significantly associated with any other potential covariates (Supplementary Table 7).

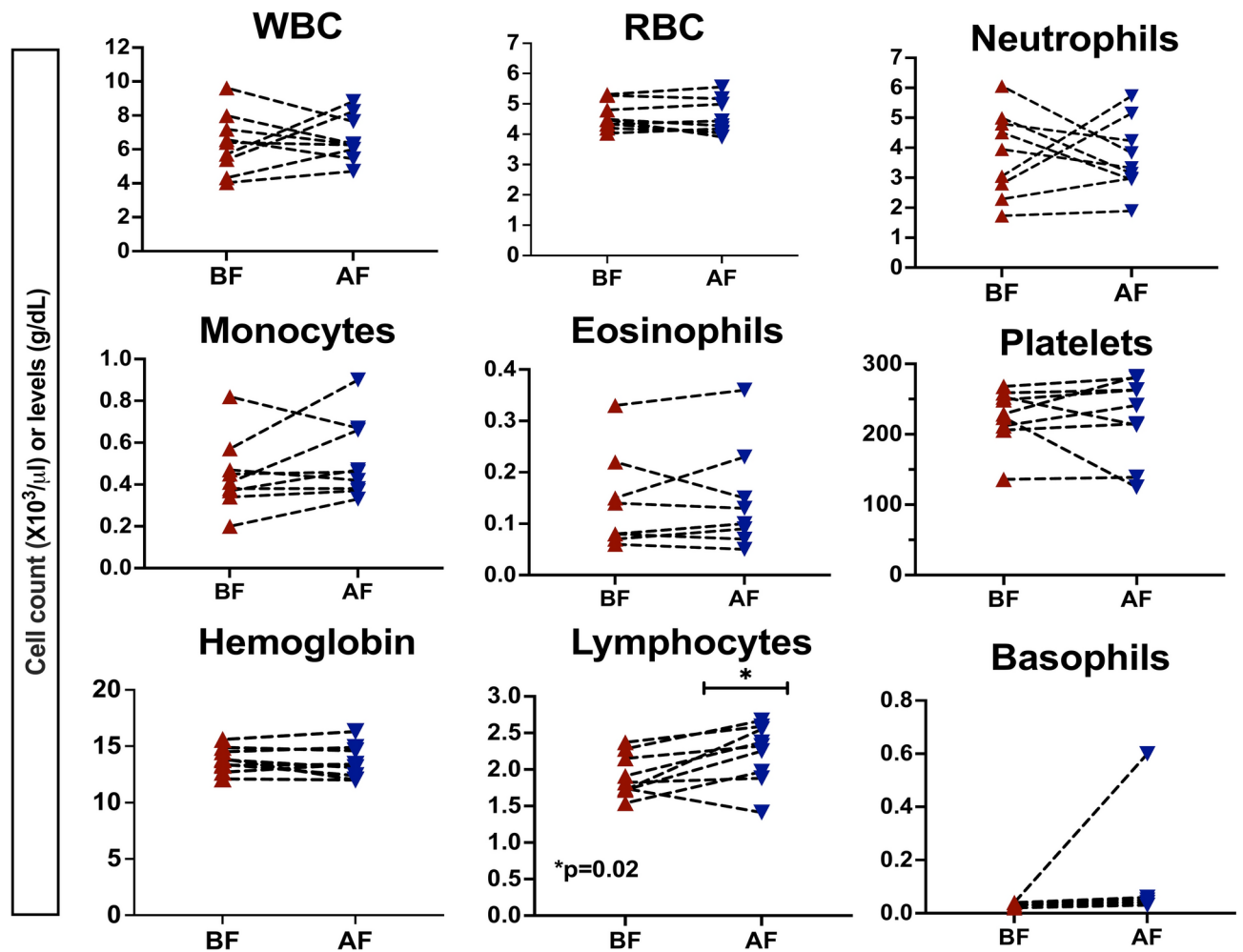


Fig. 3. Differences in blood cell count/levels in before and after flight blood samples. Each symbol represents one blood sample and the connecting line between triangle symbols indicates data are from the same individual ($n = 9$) before (BF, maroon upward facing triangle) and after flight (AF, blue downward facing triangle). Concentration for hemoglobin (g/dL) or count ($10^3/\mu\text{L}$) for White blood cell (WBC), red blood cell (RBC), neutrophils, monocytes, eosinophils, platelets, lymphocytes, and basophils is plotted on the y-axis. Asterisks indicate $p \leq 0.05$ by two-tailed paired t-test.

Alterations in DNA repair capacity (DRC) in after flight samples

DRC plays critical roles in determining whether an individual is sensitive to the cytotoxic and genotoxic effects of DNA damaging agents and the associated disease risk^{40–42}. To measure DRC in FA, in addition to the aforementioned CometChip assays, high throughput functional based FM-HCR assays were used. FM-HCR assays report the ability of live cells to repair DNA damage that alters the accuracy or efficiency of transcription of a reporter gene modified with site-specific DNA lesions^{12,28,43}. To assess DRC alterations in multiple DNA repair pathways, ten reporter plasmids (Table 1), each containing different DNA lesions (as previously described²⁸, also in methods Sect. “Assessing DNA repair capacity (DRC) in FA using high throughput fluorescence multiplex based host cell reactivation (FM-HCR) assays”, were transiently transfected into quiescent PBMCs obtained from blood samples collected before and after flight from participating FA. Specifically, plasmids with enzymatically generated DSBs were used to measure NHEJ or HR, while a plasmid with a site-specific tetrahydrofuran abasic site analog was used to measure long patch-BER (LP-BER). Plasmids bearing a single base mismatch (G: G) were used to measure MMR, and plasmids subjected to ultraviolet (UV) irradiation (UV-C, 800 J/m²) or ionizing radiation (IR) (3000 Gy, ¹³⁷Cs γ -radiation) were used to measure NER or repair of IR-induced plasmid damage, respectively. The UV dose is estimated to induce approximately 12 lesions per plasmid and was chosen based on previous host cell reactivation studies by our group and others^{12,28,44,45}. The IR dose (3000 Gy) was chosen based on previous reports that approximately 150 Gy are needed to inhibit transcription and induce 1 lesion per 30 kb⁴⁶; the transcribed region of our reporter system is 20-fold smaller (1.5 kb). In the case these six reporters, fluorescent reporter protein expression is suppressed unless repair occurs through the indicated pathways. Hence, the DRC is directly proportional to the expression of these reporter plasmids. However, four other reporters operate under a different principle based on transcriptional mutagenesis. Site-specific DNA lesions in these reporters alter the sequence of the transcribed RNA, leading to fluorescent protein expression

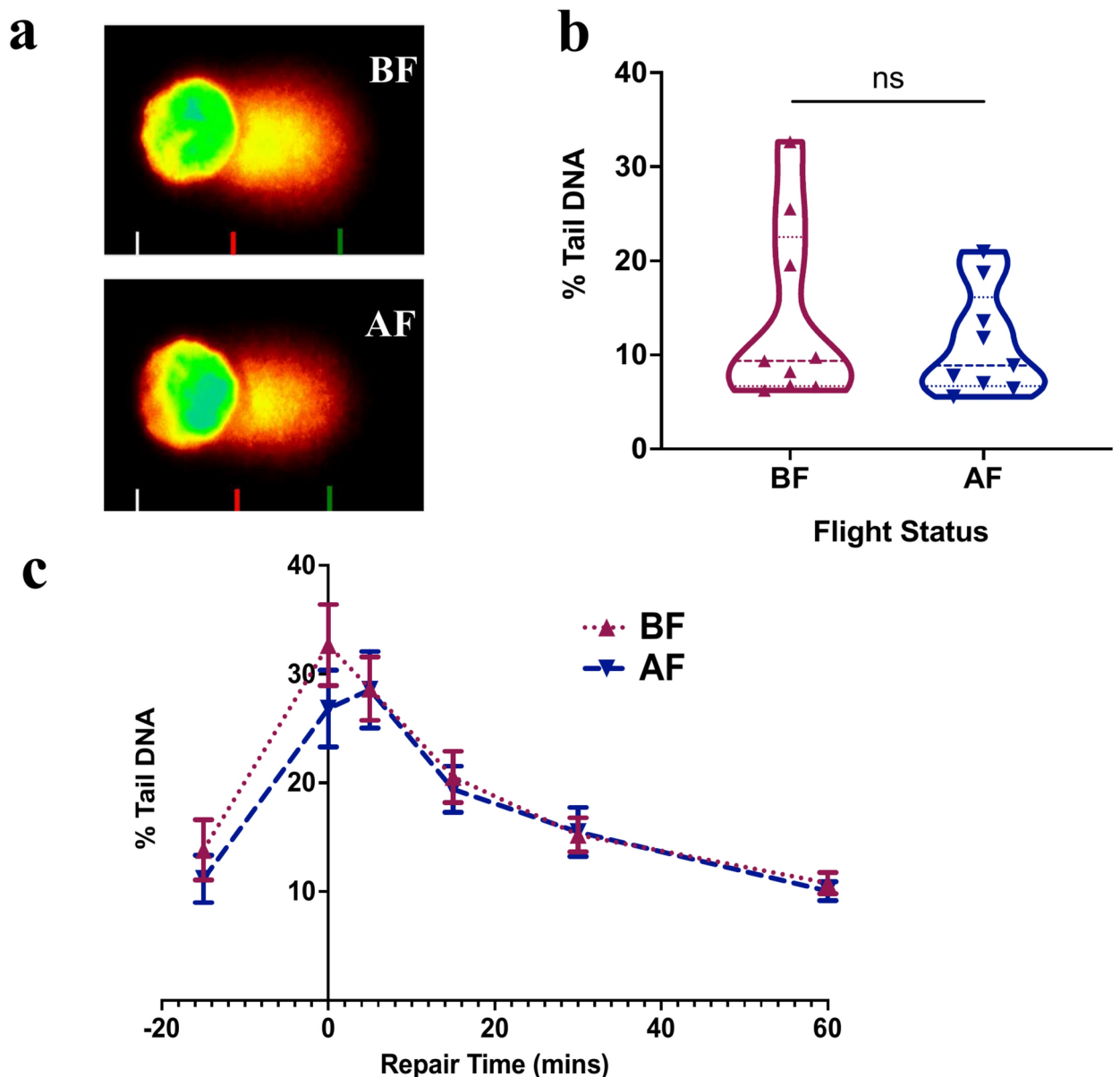


Fig. 4. Quantitation of DNA basal damage and repair kinetics using the alkaline CometChip assay. **(a)** Representative comet images of basal DNA damage (refers to basal genomic DNA damage prior to irradiation) in before (BF) and after (AF) flight sample in quiescent PBMCs. The area between the red and green lines indicates the DNA damage in tail region (% Tail DNA) that is detectable above background fluorescence. **(b)** Violin plot representing the basal DNA damage in isolated PBMCs from BF (maroon) and AF (blue) samples ($n=9$ FA). Each symbol represents the average of 5 median values from approx. 300 comets per individual at each timepoint. “ns” represent no significant difference among the samples tested using two-tailed paired t-test. **(c)** PBMCs were irradiated with 4.0 Gy of X-rays and DNA damage was measured at 0-, 5-, 15-, 30- and 60-min post irradiation along with mock irradiated (-15 min) samples obtained from FA before (BF, maroon dotted line, upward facing triangle) and after (AF, blue solid line, downward facing triangle) flight travel. The percentage of DNA in the tail region (%Tail DNA, y-axis) vs repair time (in min, x-axis) represents average repair kinetics of nine FA. Each symbol represents the average of 5 median values from approx. 300 comets each per timepoint per FA ($n=9$ FA). Error bars represent mean \pm SD.

that is suppressed when the DNA lesion is removed. Consequently, the expression of these reporter plasmids is inversely proportional to DRC. To measure the repair of damaged bases by methylpurine DNA glycosylase (MPG), a reporter plasmid with a site-specific hypoxanthine (Hx) lesion embedded opposite thymine was used. A plasmid with a site-specific uracil opposite guanine (U:G) was used to measure the removal of uracil, which can be initiated by several DNA glycosylases⁴⁷. A reporter plasmid with site-specific 8-oxoguanine opposite cytosine (8oxoG:C) reports the activity of 8-oxoguanine DNA glycosylase (OGG1) and other glycosylases that repair the same DNA lesion, including the Nei-like DNA glycosylases NEIL1 and NEIL2. Additionally, a reporter

Fluorochrome of reporter plasmid (filter, flow channel)	Undamaged cocktail (1–2)		Damaged cocktail (1)			Damaged cocktail (2)		
	Plasmid	Qty (ng)	Plasmid	Qty (ng)	Measure	Plasmid	Qty (ng)	Measure
BFP (440/50, VL-1)	pMax_BFP	30	BFP_U	20	Uracil lesion	BFP_NHEJ	50	NHEJ
AmCyan (468/98, VL-2)	pMax_AmCyan	100	pMax_AmCyan	100	Control	pMax_AmCyan	100	Control
GFP (530/30, BL-1)	pMax_GFP	10	GFP_Hx	20	Hypoxanthine lesion	GFP_THF	10	Long patch BER
mOrange (585/16, YL-1)	pMax_mOrange	20	mOrange_8oxoG-C	60	8oxoG:C lesion	mOrange_GG	20	MMR
mPlum (695/40, YL-3)	pMax_mPlum	50	mPlum_A-8oxoG	200	A:8oxoG lesion	mPlum_UVJ/m ²	100	NER
Carrier plasmid DNA (non-fluorescent)	pCX-NNX-Δ3GFP	1000	pCX-NNX-Δ3GFP	1000	—	pCX-NNX-Δ3GFP	1000	—
Undamaged cocktail 3		Damaged cocktail (3)						
BFP (440/50, VL-1)	pMax_BFP	30	pMax_BFP_IR (3000 Gy)	100	Ionizing radiation damage			
GFP (530/30, BL-1)	pCX-NNX_GFP	150	Δ5GFP_-StuI linear	150	HR			
mOrange (585/16, YL-1)	pMax_mOrange	30	pMax_mOrange	30	Control			
Carrier plasmid DNA (non-fluorescent)	pCX-NNX-Δ3GFP	1500	pCX-NNX-Δ3GFP	1500	—			

Table 1. Composition of plasmid cocktails used in this study: Five plasmid cocktails (undamaged 1–2, damaged 1, damaged 2, undamaged 3 and damage 3) of five colors (BFP, GFP, AmCyan, mOrange, and mPlum) were prepared to measure the repair capacity of cells for six DNA repair proteins/pathways. Plasmids in the pMax or pCX-NNX backbone were used for this study as in our previously published reports. The first column of the table identifies the fluorescent protein detected, bandpass filter, and flow channel as named by the software associated with the Attune NxT flow cytometer. The quantity (qty) of each plasmid (in nanograms) is indicated. pMax_AmCyan (in damaged cocktail 1 and damage cocktail 2) and pMax_mOrange (in damaged cocktail 3) were used as transfection controls.

plasmid with undamaged adenine in the transcribed strand opposite to 8oxoG (A:8oxoG) was used to measure the activity of the mutY DNA glycosylase (MUTYH). Each cocktail included a damage-free control to normalize for transfection efficiency, and the reporter expression was calculated by normalizing expression of damage containing reporter plasmids to separately transfected damage-free counterparts as described previously^{12,28,48}. Detailed information on obtaining the final DRC readout using flow cytometry (Supplementary Figs. 6a, b and 7) and the preparation of the reporter plasmids is presented in the methods section and previous reports^{28,30}.

We did not observe a significant difference in transfection efficiency between BF and AF samples (Supplementary Fig. 6c), but FM-HCR revealed alterations in DRC for some pathways comparing BF and AF samples after adjusting for multiple testing (Figs. 5 and 6). We observed a significant decrease in repair (increase in reporter expression, adjusted $p=0.035$) of the oxidative DNA lesion 8oxoG:C in the AF samples as compared to BF (Fig. 5a, b). Additionally, we observed a significant decrease (adjusted $p=0.025$) in repair capacity for IR-induced plasmid damage in the AF samples (Fig. 6a, b). Together, the FM-HCR data are consistent with the CometChip data in two respects. First, we observed a maximum level of strand breaks immediately after irradiation (at t_0) in the BF samples, but at 5 min post-irradiation (at t_5) in the AF samples (as seen in Supplementary Fig. 3; measured using CometChip assay), consistent with slower initiation of BER that was detected by the FM-HCR assays (Fig. 5a, 8oxoG:C reporter expression). Second, we observed a trend towards overall slower resolution of SSBs in the CometChip assay, consistent with the slower completion of repair for the IR-induced plasmid damage measured using FM-HCR assay.

Correlations between markers of genomic instability and immunophenotype

Some notable correlations were observed among DNA repair assays and between DNA repair assays and other measured parameters. To prevent false positives and ensure that true associations aren't overlooked, we applied multiple testing to the Pearson's correlation coefficient by adjusting the false-discovery rate (FDR) to 0.01 (as discussed in method Sect. "Statistical analysis and graphical representations") resulting in fewer associations reaching significance. For instance, a significant positive correlation was observed between eosinophil counts in the BF vs AF samples (adjusted $p=0.0002$) (Supplementary Fig. 8a, Supplementary Table 3). On comparing the basal damage and repair rate (K) in BF samples (Supplementary Fig. 8b; Supplementary Table 4), we also observed a significant correlation between the basal damage and hypoxanthine (Hx) adduct repair (adjusted $p=0.003$). We observed a significant positive correlation between the repair rate (K, as measured by CometChip) vs repair capacity for the IR-induced plasmid damage (IR) as measured by FM-HCR (adjusted $p=0.004$). On correlating the DRC measured using FM-HCR assays, we observed significant correlation of 8oxoG:C lesions (adjusted $p=0.0015$) in BF vs AF samples and NHEJ DRC activity vs basophil counts (adjusted $p=0.002$) (Supplementary Fig. 9; Supplementary Table 5).

We expanded our correlation analysis to include biological variables such as immunophenotype (Supplementary Table 6); basal DNA damage and repair rate obtained from CometChip assay (Supplementary Table 7); DRC using FM-HCR assays (Supplementary Table 8)] versus demographic variables (age, sex, BMI); and flight-related parameters (flight duration between blood draws, years of employment duration as a FA, and sleep deprivation in days) obtained from participant questionnaires. Despite the small sample size, after

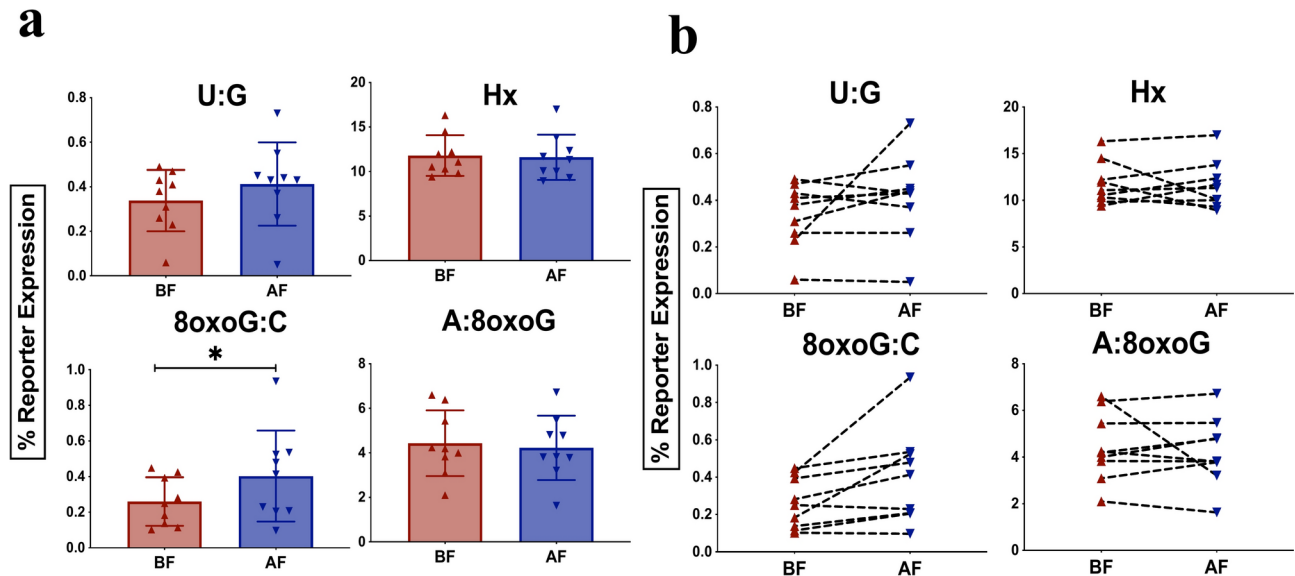


Fig. 5. Repair activity of DNA glycosylase-based lesion repair in nine FA. **(a)** DNA glycosylase-dependent initiation of BER was measured for uracil (U:G)-, hypoxanthine (Hx:T)-, 8oxoG:C-, and A:8oxoG lesions. For these assays, the unrepaired DNA lesion results in a transcriptional error that leads to fluorescent reporter expression. Consequently, reporter expression is inversely proportional to repair capacity. % Reporter expression is plotted on the y-axis versus the flight status (before flight, BF, maroon bar) or (after flight, AF, blue bar) on x-axis. Each symbol represents a single FM-HCR measurement from one FA. Error bars represent mean \pm SD. Asterisks indicate $p \leq 0.05$ by two-tailed paired t-test. **(b)** Data from panel A are replotted with a connecting line between triangle symbols to indicate data from the same individual before (BF, maroon upward facing triangle) and after flight (AF, blue downward facing triangle).

multiple comparison correction (FDR, adjusted $p \leq 0.05$), we observe some modest correlations in AF samples (Supplementary Tables 6–8). For example in AF samples (Supplementary Table 6), eosinophil counts correlated with flight duration (adjusted $p = 0.02$) and years of employment (adjusted $p = 0.01$); basophil counts (adjusted $p = 0.02$) and Hb level (adjusted $p = 0.04$) correlated with participant sex; and platelets counts correlated sleep deprivation (adjusted $p = 0.002$). No significant correlations were found between these potential confounding factors and basal DNA damage and repair rate (K) measured using CometChip assay (Supplementary Table 7). Additionally, in AF samples, some significant correlations were observed between DRC and other variables (Supplementary Table 8), such as BMI with Hx:T lesion repair capacity (adjusted $p = 0.005$) and NER capacity with sleep deprivation (adjusted $p = 0.008$). While there is no apparent intuitive explanation for some of these relationships, and they should be interpreted with caution given the small sample size, future studies may explore whether they represent previously unknown relationships^{29,32,49–56}. Finally, for parameters that were significantly different comparing BF and AF samples [lymphocyte counts (Fig. 3, Supplementary Table 9), repair of 8oxoG:C (Fig. 5, Supplementary Table 10), and repair of IR-induced plasmid damage (Fig. 6, Supplementary Table 11)], we tested whether the change in those parameters correlated with changes in other parameters. Correlation between the changes in repair of IR induced plasmid damage was significant with changes in NHEJ repair capacity (adjusted $p = 0.002$) (Supplementary Table 11), consistent with role of NHEJ in repairing one or more DNA lesions induced by IR⁵⁷. Due to the varied response in neutrophil counts among AF samples, where some samples ($n = 4$) showed an upward trend while others ($n = 5$ FA) exhibited a decline, we performed the correlation analysis to assess their connection with changes in other measured biological endpoints. We observed a strong correlation between neutrophils and white blood cells (adjusted $p < 0.0001$) only, which was expected since neutrophils are a subset of white blood cells (Supplementary Table 12). Otherwise, no significant associations were observed.

Overall, our findings indicate changes in blood cell count, heterogeneity in repair kinetics, and reduced activity in repairing oxidative and IR-induced lesions in AF travel samples in the studied FA.

Discussion and conclusions

FA are a relatively understudied group of workers who are more susceptible to chronic illness due to their profession compared to the general population¹. Mounting evidence from epidemiological studies has documented elevated cancer and other diseases in FA^{4,7,8,33,35,58,59}, but the etiology is unknown. Exposure to a complex mixture of potentially mutagenic agents from various sources during the flight travel may challenge FA health^{33,34}. Among these occupational exposures, cosmic ionizing radiation (CIR) stands out as a DNA damaging carcinogen³. The National Council on Radiation Protection and Measurements (NCRP) has formally classified FA as radiation workers, receiving an average annual dose of 3.07 mSv, which is five times the average dose (0.59 mSv) of U.S Department of Energy radiation workers⁶⁰. The inter-individual variation we observe with

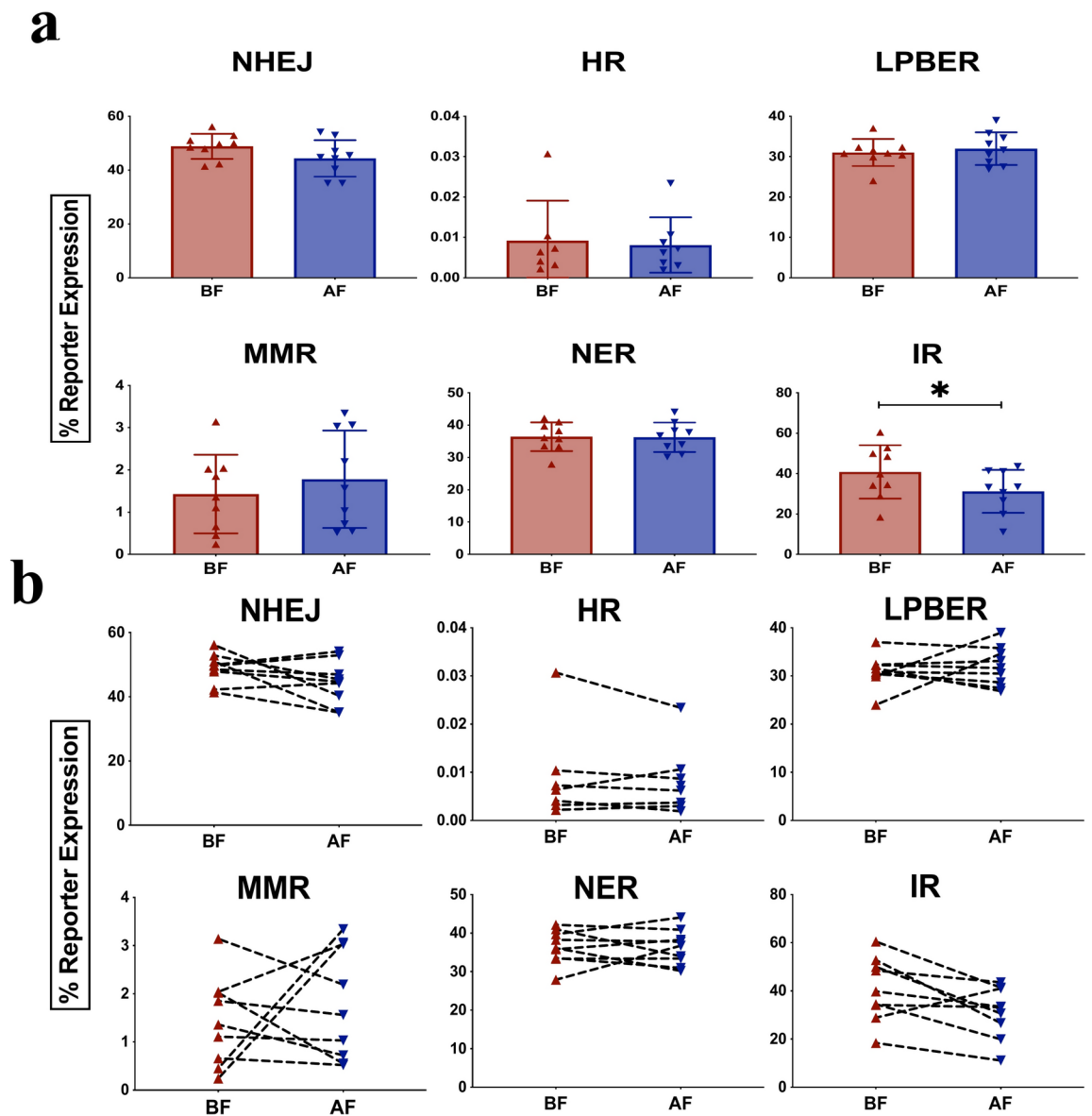


Fig. 6. Repair activity of various DNA repair pathways in nine FA. **(a)** Graphs represent % reporter expression for non-homologous end joining (NHEJ), homologous recombination (HR), long patch base excision repair (LPBER), mismatch repair (MMR), nucleotide excision repair (NER) and IR-induced plasmid damage (IR) on y-axis against flight status [(before flight, BF, maroon bar) and (after flight, AF, blue bar)] on x-axis. The reporter expression for these plasmid DNA is directly proportional to the repair capacity. Each symbol represents a single FM-HCR measurement from one FA. Error bars represent mean \pm SD. Asterisks indicate $p \leq 0.05$ by two-tailed paired t-test. **(b)** Line connecting between the triangle symbols of BF (maroon upward facing triangle) and AF (blue downward facing triangle) represent reporter expression of each FA for NHEJ, HR, LPBER, MMR, NER, and IR-induced plasmid damage repair activity.

respect to repair of IR-induced genomic DNA damage could contribute to the heterogeneity in radiosensitivity reported by others^{3,42,55,61}. Individual FA's exposure levels can vary considerably depending on flight parameters including the area of the aircraft in which they work⁶², cruising altitude and latitude^{60,63–66}, and solar activity^{63,64}. Studies have associated CIR exposure among FA with increased cancer incidence and mortality and diminished reproductive health^{3,4}. Although in-flight radiation exposure is expected to be on the order of 20–40 micro Sieverts and thus results in far less DNA damage than is induced by endogenous agents but the type of damage induced may be more dangerous; as even low doses of radiation can influence blood cell counts and DRC^{61,67–69}. Research using models of the individual components of CIR has been conducted using in vitro human cell cultures^{3,70–72}, and the data have been extrapolated from high-dose radiation exposure to assess the effects of low-dose/dose rate exposure in humans^{73,74}. However, caution is needed when applying these findings to the FA cohort, as they are exposed to multiple mutagenic factors that may have additive effects alongside chronic intermittent low-dose CIR exposure⁴. Additionally, their exposure spans a range of radiation types which adds to

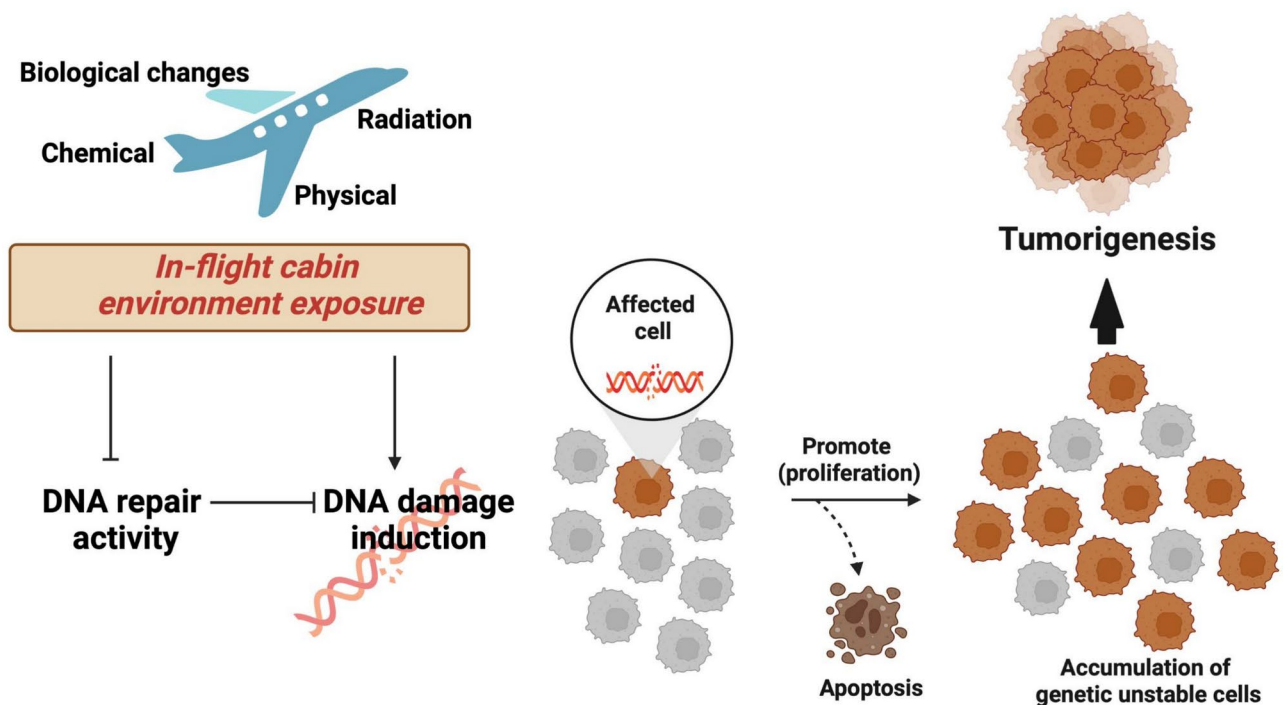


Fig. 7. Proposed model where DNA damage promoted by in-flight exposures is a key biological mechanism underlying cancer in FA. Exposure of FA to radiation, mutagenic chemicals, various physical and biological stressors cause DNA damage. The same exposures may inhibit DNA repair, leading to further accumulation of DNA damage and ultimately cancer-causing mutations.

the potential complexity of biological effects^{66,75,76}. The absence of biological measurements poses a significant barrier to formulating regulatory policies, particularly concerning the potential impact on genomic integrity of individuals working as flight crew. Complexity arises from the potential of exposure to a diverse mixture of mutagens, coupled with variability in parameters such as aircraft type, cruising altitude, travel duration, flight path (i.e. circumpolar), and time zone differences. This complexity poses challenges in conducting a cohort studies aimed at understanding the basis for elevated cancer risk in FA. Our repeated measures study design presents an opportunity to identify functional biomarkers that measure the effects of flight travel at intra-individual level, thus overcoming some of the aforementioned complexity.

Since genomic instability and inflammation are interrelated hallmarks of carcinogenesis^{49,77–80}, we focused our analyses on markers of DNA damage and immune disruption. The complex mixture of occupational exposures encountered by FA may trigger systemic immune responses, damage DNA, or interfere with DNA repair mechanisms (Fig. 7). Individuals respond differently to DNA damaging agents, due to differences in genetics, age, sex, BMI, lifestyle and other factors^{29,32,50–56}. Studies have shown that the demanding workload of FA, characterized by irregular working hours and exposure to the unique in-flight cabin environment, including disruption of circadian rhythm, can directly or indirectly affect their performance, sleep patterns, health, and social and family life⁸¹. FA have reported experiencing fatigue, excessive sleepiness, shift-work disorder, insomnia, unhealthy lifestyle habits, and depression³⁶. Increasing age and longer employment duration as a FA are key factors associated with prolonged exposure to mutagens, such as secondhand tobacco smoke^{1,7,33}. Additionally, FA whose employment began before the mandated smoke-free environment onboard aircraft exhibited higher rates of chronic bronchitis and respiratory symptoms³³. Employment duration also serves as an indicator of exposure to other carcinogens in flight, which may include CIR, solar particles, and other agents^{33–35}. We explored correlations between several variables, including age, sex, BMI, flight duration, years of employment as a FA and sleep deprivation and DNA damage and repair (Supplementary Table 7). While these associations generally did not reach significance in our small samples size, our findings lay a foundation for follow up in future larger studies that can determine their relevance for inclusion as covariates in statistical models. To understand how occupational exposures affect FA, detailed individualized biological data are needed. We have measured blood cell composition and quantitated DNA damage and repair activity in the participating FA from the time of departure from Boston airport and upon return to Boston. Although our pilot study has a very small sample size, we observe a significant increase in lymphocyte and basophil counts, which is often associated with inflammatory responses to allergens or infections, circadian rhythm disruption, and other stressors^{38,39,82–86}. Consistent with such an inflammatory response, a previous study found elevated markers of oxidative stress when comparing pre- and post-flight blood samples collected from pilots⁸⁷. Our data are also consistent with previous reports of changes in blood cell counts following air travel⁸⁸. Of note, blood cell composition can vary with age, sex, race, BMI, infection, vaccination and many other factors⁴⁹, such as the physical forces caused by

vibrations during flight travels⁸⁶. The variability in immune status of FA may influence the link between the DRC and the associated cancer or disease risk which requires further investigation.

The 8oxoG:C lesion is produced endogenously as a result of oxidative stress and is predominantly excised *via* BER initiated by the 8-Oxoguanine (OGG1) glycosylase. FA may be exposed to multiple mutagenic factors during flight travel, including radiation and other environmental or in-flight cabin stressors, which can also contribute to the formation of 8oxoG:C lesion^{3,89,90}. Diminished repair of this lesion in AF samples is notable, given studies that have linked diminished OGG1 activity to an elevated risk of cancer^{91,92}. Previous studies in flight crew cohorts have also documented subtle increase (did not attain significance) in oxidative damage or strand breaks^{87,93,94}, micronuclei formation⁹⁵, and chromosomal aberrations^{93,95–99}. Strikingly, our data suggest a decrease in repair of IR-induced damage and 8oxoG:C lesion repair efficiency as measured by both CometChip and FM-HCR assays, although the trend for CometChip data did not reach statistical significance. Our data suggest that FA may be more vulnerable to mutagen exposure due to suppression of the mechanisms that protect against DNA damaging agents. While our findings need to be confirmed in larger studies, the difference in AF DRC (between 20 and 50%) is of concern since differences of this magnitude have been associated with odds ratios in the range of 2–7 for several cancers^{41,42,100}. Taken together, our data suggest that air travel simultaneously promotes inflammatory processes that may increase oxidative DNA damage and suppresses the protective processes that repair DNA damage.

Several strengths and limitations of our study should be taken into consideration when interpreting our results and planning future research. Leveraging emerging high throughput technologies, a functional screen using FM-HCR assays was utilized to measure DRC at the individual level⁴¹. These assays are highly validated and have been applied in a wide range of cell lines and mitogen stimulated primary human cells^{12,28,30,31,42,43,48}. The application of FM-HCR assays in unstimulated quiescent lymphocytes is an important technical advance because it shortens the time in culture and eliminates potential sources of error introduced by mitogenic stimulation. The assay provides a comprehensive analysis of DRC in all major repair pathways, enabling the discovery of potential multi-pathway signatures of cancer risk. We note that cancer risk models that combine multiple functional assays outperform models that use polygenic risk scores or functional data for a single DNA repair pathway¹⁰¹. While plasmid-based assays cannot completely recapitulate the complex chromatin environment of genomic DNA, our FM-HCR data are consistent with our data obtained from CometChip assays, which are not subject to this limitation. PBMCs have been used as a representative cell type when investigating cancer risk in populations^{102–104}. Our study depends on PBMCs to serve as a surrogate cell type for other tissues where cancers develop¹⁰⁰. While DRC varies by cell type, PBMCs have been used widely for this purpose by others^{55,67,68,105–109}. Studies have found increases in chromosomal aberrations and DNA damage in PBMC obtained from flight crew^{93,98}. Those findings indicate genome instability and are in accordance with our CometChip assay findings.

Previous studies have also found evidence for long-term adaptive responses^{52,55,110}, where DRC might appear to be higher among those with longer service as flight crew. Given our small study, we were not able to evaluate the relationship between DRC and cumulative exposure rigorously, and future larger studies are needed to investigate this possibility. Understanding the stability of DRC over time, particularly with repeated measurements, will be crucial for the interpretation of studies investigating within-person changes in DRC. Larger studies are also needed to enable rigorous statistical analyses that adjust for the many potentially confounding variables that may influence DNA damage and repair in FA. These include demographics, flight schedules, aircraft model, flight paths, seasonal effects, lifestyle, health status, direct individual radiation dosimetry measurements, and the washout period for flight-associated genotoxic events. Given the variability in flight schedules and the unique demands associated with work as flight crew, the selection of an appropriate control group for comparison requires well-defined criteria to minimize the risk of bias.

Future studies will benefit from larger samples sizes and more detailed datasets than we have reported here. Precise dosimetry is essential for accurate assessments of mutagenic exposures in the flight cabin environment. Currently available indirect measurements of radiation exposure in flight crew using computational models shows variability depending on the specific CIR components measured^{3,4}. Future research should focus on improving accuracy in estimating CIR exposure for flight crew by incorporating actual flight data, considering factors such as route, duration, latitude, altitude, solar cycle variations and others. This research is crucial for developing individualized dosimetry and identifying biomarkers to assess radiation related health risks in flight crew. Furthermore, estimating the washout period for flight-induced DNA damage and repair alterations can help establish the time intervals needed between flight and return to baseline for FA based on individual's DRC. This will provide a critical data to give an estimation of effect sizes to determine optimal experimental conditions. An ideal study would include detailed multi-omics analysis of biological samples including genotyping, gene expression; additional markers of genome instability such as clonal hematopoiesis and telomere length; and additional markers of immune disruption, such as immunophenotyping by CyTOF and analysis of cytokines in blood plasma. These data will be critical for developing statistical models aimed at estimating cancer risk on an individualized basis for FA.

Our finding of changes in differential blood cell counts and disruption of DNA repair mechanisms sheds new light on the biological effects of air travel in FA. Our study serves as a valuable starting point for broader future research by demonstrating the feasibility of measuring DNA damage and repair in biological samples from FA and lending support for a model in which occupational exposures cause genome instability and immune disruption to contribute to excess cancer risk in FA (Fig. 7). By successfully employing FM-HCR in resting lymphocytes and immunophenotyping on a small scale, we established a reliable framework that can be scaled up in future research and in space exploration for astronauts. Furthermore, our preliminary findings provide a sound scientific basis to justify more extensive studies, thereby paving the way for comprehensive exploration of genome integrity and immune function in FA. Such studies will be instrumental in identifying strategies for safeguarding FA health¹¹¹.

Methodology

Sample collection and differential blood cell count

The Institutional Review Board at Harvard T H Chan School of Public Health (IRB18-01845) approved all experimental procedures and methodologies (all was performed in accordance with relevant guidelines and regulations), including written informed consent and study advertisements for participant recruitment. Written informed consent was obtained from FA before their participation in the study. Demographic details including lifestyle behaviors were collected via IRB approved questionnaires (Supplementary doc.1). FA provided their schedules 2 weeks in advance to coordinate the timing of blood draws. Approximately 20 ml of venous blood was collected in BD vacutainers coated with K₂EDTA (Cat no. 367863) from nine FA, before (5–12 h before departure time from Boston Logan International airport) and on return of (at the earliest convenience after landing, but no later than 1.5 h) their flight at Boston Logan International Airport in Boston, Massachusetts, USA. On average, time between blood collection to start the experimental procedure in lab ranged between 30 and 45 min. Differential blood cell counts (including white blood cell, red blood cell, hemoglobin, neutrophil, eosinophil, basophil, total lymphocytes, monocytes, platelets and others) using 100 µl of blood sample were recorded at the Pathology Department of Brigham and Women's Hospital, Boston, Massachusetts, USA.

Peripheral blood mononuclear cells (PBMCs) isolation from collected blood

PBMCs were isolated from blood samples by density gradient centrifugation using Lymphoprep (StemCell Technologies, Cat no. 07811) in SepMate tubes (StemCell Technologies, Cat no. 85460). Each volume of blood was mixed an equal volume of 1X Dulbecco's phosphate buffer saline (DPBS, Gibco, Cat no. 14190144) containing 2% fetal bovine serum (FBS, Gibco, Cat no. 10437028) before adding to the SepMate tubes. PBMC isolation was carried out according to the manufacturer's protocol¹¹². A trypan blue dye exclusion assay was used to measure viable cell numbers using a Vi-Cell XR Cell counter, Beckman Coulter (Cat no. TOC Analyzer QbD1200). Isolated PBMCs viability above 90% was considered acceptable for downstream analysis, based on previous optimization of our high-throughput CometChip and FM-HCR assays. Subsequently, the isolated PBMCs were divided into two aliquots (2X10⁶/aliquot) for further experimental analysis.

Quantification of DNA damage and repair kinetics using high-throughput CometChip assay

As described previously^{30,113}, alkaline CometChip assay were performed to measure DNA damage and repair kinetics in freshly isolated PBMCs. PBMCs in the amount of 10,000 cells per 100 µl/well were loaded into the 96 well plate of 1% normal melting agarose (Gold Biotechnology, Cat no. A-201-100). After loading and settling by gravity for 15 min, the chip was washed with 1X DPBS to remove excess cells and then overlaid with 1% low melting agarose (Gold Biotechnology, Cat no. A-204-100). For repair kinetics, the CometChip was then divided into different repair timepoints and were irradiated at 4.0 Gy of X-rays on ice at a dose rate of 1.0 Gy/min (RS2000 Biological System, USA). DNA damage was measured at 0-, 5-, 15-, 30- and 60- min post-irradiation. A mock-irradiated chip (0 Gy or -15 min) and 4.0 Gy (0 min, *t*₀) chips were immediately immersed in cold alkaline lysis buffer (2.5 M NaCl [Millipore Sigma, Cat no. S9888], 100 mM Na₂EDTA [Millipore Sigma, Cat no. E9884], 10 mM Tris, pH 9.5 [Millipore Sigma, Cat no. 10708976001] with 0.5% Triton X-100 [VWR, Cat no. 97062-208]) and incubated overnight at 4 °C. Remaining chips were immersed in pre-warmed RPMI 1640 medium (Gibco, Cat no. 11875085) supplemented with 20% FBS at 37 °C, 5% CO₂ in humidified incubator. Thereafter, the chips at the respective repair timepoints were transferred into the cold alkaline lysis buffer for overnight incubation at 4 °C. The chips were then treated for alkaline unwinding buffer (0.3 M sodium hydroxide [VWR, Cat no. 97064-486] and 1 mM Na₂EDTA in distilled water) followed by electrophoresis (25 V, 300 mA, 20 min) at 4 °C. Next, neutralization was performed using 0.4 M Tris-HCl buffer, pH 7.5 followed by staining with 1X SYBR gold nucleic acid gel stain (Invitrogen, cat. no. S11494). Fluorescence images of the comets were captured at 10X magnification using an inverted LED fluorescence motorized microscope (Zen 3.2 pro blue edition, Zeiss Apotome 2, Carl Zeiss microscopy, GmbH). Comet images were analyzed using Trievgen comet analysis software (R&D systems, MN, USA). Median of approximately 1500 cells were scored to calculate the percentage of DNA in the comet tail region (% Tail DNA) for a particular timepoint, which represents the level of DNA damage. Quality check includes exclusion of comet tail overlaps, cells at the edges of chips, apoptotic cells or cells for which % Tail DNA exceeds 80% or comet morphology exhibiting a “halo” or “hedgehog” appearance, indicating extensive DNA fragmentation and dispersion¹¹⁴. For correlation analysis, repair rate (K) was quantified [generated by Prism 8.1 software] using an exponential decay model assuming DNA damage follows a first-order kinetics¹¹⁵.

Assessing DNA repair capacity (DRC) in FA using high throughput fluorescence multiplex based host cell reactivation (FM-HCR) assays

- i) *Plasmid reporter and cocktail preparation* DRC was assessed in the isolated quiescent PBMCs obtained from the FA blood samples (before and after flight) using FM-HCR assays. Reporter plasmids with DNA lesions were prepared and the assays performed following procedures described briefly herein; more detailed methodology is described in previous publications^{12,28,29}. Table 1 provides comprehensive information about the plasmid fluorochrome, the corresponding DNA lesion, the DNA pathway being measured, and the composition of each plasmid cocktail. To normalize for transfection efficiency, each cocktail included a transfection control, namely pMax_AmCyan (for undamaged 1–2, damage 1, damage 2) or pMax_mOrange (for undamaged 3, damage 3). Additionally, pCX-NNX-Δ3GFP¹¹⁶, a plasmid originally developed for use in homologous recombination assays, was used here as a carrier DNA that does not express a fluorescent protein and enhances transfection efficiency.

- ii) *Transfection of quiescent PBMCs with plasmid cocktails* Isolated PBMCs were washed with 1X DPBS containing 2% FBS and collected by centrifugation at 400 g for 8 min. For each transfection, 2×10^5 cells were resuspended in buffer T and transfected using Neon™ Transfection System 10 μ L Kit (ThermoFisher Scientific, Cat no. MPK1096). Six aliquots were prepared, each with 12.5 μ L buffer T (provided by manufacturer for transfection). Five aliquots were used for transfection with a plasmid cocktail, and one aliquot was kept as an un-transfected negative control. 1.5 μ g of plasmid cocktail (as specified in Table 1) was mixed with the cells suspended in T buffer. Electroporation was carried out using a Neon Transfection System (ThermoFisher Scientific, Cat no. MPK5000) with single 20 ms pulse at 2250 V. These conditions were selected based on optimal transfection efficiency (23–35%) and viability (80–89%) post transfection. Single color control transfections (200 ng of damage-free reporter plasmid) were carried out to provide samples for adjusting gating, voltage setting, and compensation. Post transfection, the cells were immediately cultured in pre-warmed RPMI 1640 containing 20% FBS in 24 well tissue culture plate for 18 h at 37 °C, 5% CO₂ in humidified incubator.
- iii) *Flow cytometry and analysis* At 18 h post-transfection incubation, the cells were thoroughly resuspended and transferred to flow tubes for flow cytometry analysis using Attune™ NxT Flow cytometer (ThermoFisher Scientific, Cat no. A29001). Cell debris and doublets were excluded based on their side scatter and forward scatter properties (Supplementary Fig. 5a, b). Data were analyzed in the Attune™ NxT software and Micro-soft Excel, as described previously²⁸. For quality control purposes, ≥ 30 fluorescent positive events were required as the minimum threshold for data inclusion in our FM-HCR analysis (Supplementary Fig. 6). Normalization for transfection efficiency and calculation of DRC were performed as described previously^{28,29}. Briefly, after normalizing to a transfection control, reporter expression from damage-containing reporter plasmids was normalized to the expression from undamaged plasmids expressing the same fluorescent reporter protein and reported as percent reporter expression. The assays for NHEJ, long patch-BER, MMR, NER, HR, and IR-induced plasmid damage (IR) yield reporter expression that is directly proportional to repair capacity. For these assays, % reporter expression approximately represents the percentage of plasmids from which the DNA damage has been removed. The assays for repair of the U:G (uracil) lesion, Hx:T (hypoxanthine) adduct, 8oxoG:C lesion, and A:8oxoG lesion yield a fluorescent signal that is inversely proportional to the repair capacity because they report the presence of the DNA lesion through transcriptional mutagenesis that occurs during lesion bypass by RNA polymerase. For these assays, % reporter expression approximately represents the percentage of plasmids in which the DNA damage remains.

Statistical analysis and graphical representations

Figures 1a and 7 and Supplementary Fig. [3, except the comet images] were generated with Biorender.com. All statistical analysis (except for correlation analysis) and graphical presentation were performed using PRISM 8.1 software. For the CometChip assay, two tailed -paired t test with Bonferroni multiple testing correction (conservative approach to reduce false positives) was used to compare statistical significance in BF and AF samples. The DNA repair half time $t_{1/2}$ was calculated for repair kinetics using nonfit exponential one phase decay (due to smaller sample size and for effectively capturing the biological trend of initial damage accumulation in AF sample followed by a single-phase repair process, ensuring interpretability without overfitting). When comparing the differential blood cell counts and FM-HCR assays in BF versus AF samples, two tailed paired t test with Bonferroni multiple testing correction was used to determine the statistical significance. The threshold for statistical significance was set at $p \leq 0.05$. For correlation analysis, Z-score (log) transformed reporter expression in % was obtained. Correlation analysis was conducted in R¹⁷ and evaluated by Pearson's correlation coefficient and multiple testing was adjusted by false-discovery rate (FDR). FDR was used to control the proportion of false positives rather than eliminating them entirely, ensuring that meaningful associations are not overlooked while still maintaining statistical rigor (adjusted $p \leq 0.05$). Correlation matrix and Joy plot figures were created using corrplot¹¹⁸ R packages (version 0.92). For outlier testing¹¹⁹, datapoints were converted to Z-scores. Data points with Z-scores greater than 3 or less than -3 would be considered outliers and such cases were not seen in the current dataset (Supplementary Fig. 10).

Significance statement

Studies show that flight attendants face increased cancer risk and/or reproductive health issues due to air travel which might potentially be linked to DNA damage and impaired repair mechanisms. We have measured DNA damage and repair capacity in flight attendants before and after flight travel. Preliminary results from our pilot study show changes in blood cell counts and reduced repair efficiency post-flight. Combining mechanistic insights into genome integrity within larger epidemiological cohorts could provide a better understanding of the biological factors connecting flight attendants' air travel-related exposures and health outcomes. This approach could help inform strategies to protect their health in the long term.

Data availability

All data supporting the findings of this study are available in the manuscript. Additional data and materials are available from the corresponding author upon reasonable request and also available in the main text or the supplementary materials. R scripts used for analyses will be made public upon acceptance on Github.

Received: 21 January 2025; Accepted: 15 April 2025

Published online: 13 May 2025

References

- McNeely, E. et al. The self-reported health of U.S. flight attendants compared to the general population. *Environ. Health* **13**, 1–11 (2014).
- Burdon, J. et al. Health consequences of exposure to aircraft contaminated air and fume events: A narrative review and medical protocol for the investigation of exposed aircrew and passengers. *Environ. Health* **22**, 1–29 (2023).
- Toprani, S. M., Scheibler, C., Mordukhovich, I., McNeely, E. & Nagel, Z. D. Cosmic ionizing radiation: A DNA damaging agent that may underly excess cancer in flight crews. *Int. J. Mol. Sci.* **25**, 7670 (2024).
- Scheibler, C. et al. Cancer risks from cosmic radiation exposure in flight: A review. *Front. Public Health* **10**, 947068 (2022).
- Ballard, T. J. et al. Integrating qualitative methods into occupational health research: A study of women flight attendants. *Occup. Environ. Med.* **61**, 163–166 (2004).
- Griffiths, R. F. & Powell, D. M. C. The occupational health and safety of flight attendants. *Aviat. Space Environ. Med.* **83**, 514–521 (2012).
- McNeely, E., Mordukhovich, I., Tideman, S., Gale, S. & Coull, B. Estimating the health consequences of flight attendant work: Comparing flight attendant health to the general population in a cross-sectional study. *BMC Public Health* **18**, 1–11 (2018).
- Rafnsson, V., Tulinius, H., Jónasson, J. G. & Hrafnkelsson, J. Risk of breast cancer in female flight attendants: A population-based study (Iceland). *Cancer Causes Control* **12**, 95–101 (2001).
- Buja, A. et al. Cancer incidence among male military and civil pilots and flight attendants: An analysis on published data. *Toxicol. Ind. Health* **21**, 273–282 (2005).
- McNeely, E. et al. Cancer prevalence among flight attendants compared to the general population. *Environ. Health* **17**, 1–9 (2018).
- Jackson, S. P. & Bartek, J. The DNA-damage response in human biology and disease. *Nature* **461**, 1071–1078 (2009).
- Nagel, Z. D. et al. Multiplexed DNA repair assays for multiple lesions and multiple doses via transcription inhibition and transcriptional mutagenesis. *Proc. Natl. Acad. Sci. U. S. A.* <https://doi.org/10.1073/pnas.1401182111> (2014).
- Nelson, B. C. & Dizdaroglu, M. Implications of DNA damage and DNA repair on human diseases. *Mutagenesis* **35**, 1–3 (2020).
- Jeggo, P. A., Pearl, L. H. & Carr, A. M. DNA repair, genome stability and cancer: A historical perspective. *Nat. Rev. Cancer* **16**, 35–42 (2016).
- Tubbs, A. & Nussenzweig, A. Endogenous DNA damage as a source of genomic instability in cancer. *Cell* **168**, 644–656 (2017).
- Brown, J. S., Sundar, R. & Lopez, J. Combining DNA damaging therapeutics with immunotherapy: More haste, less speed. *Br. J. Cancer* **118**, 312–324 (2018).
- Lindahl, T. & Barnes, D. E. Repair of endogenous DNA damage. *Cold Spring Harb. Symp. Quant. Biol.* **65**, 127–134 (2000).
- Sancar, A., Lindsey-Boltz, L. A., Ünsal-Kaçmaz, K. & Linn, S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem.* **73**, 39–85 (2004).
- Hakem, R. DNA-damage repair; the good, the bad, and the ugly. *EMBO J.* **27**, 589 (2008).
- Chatterjee, N. & Walker, G. C. Mechanisms of DNA damage, repair, and mutagenesis. *Environ. Mol. Mutagen.* **58**, 235–263 (2017).
- Wallace, S. S. Base excision repair: A critical player in many games. *DNA Repair (Amst)* **19**, 14–26 (2014).
- Kusakabe, M. et al. Mechanism and regulation of DNA damage recognition in nucleotide excision repair. *Genes Environ. Off. J. Japn. Environ. Mutagen. Soc.* **41**, 1–6 (2019).
- Davis, A. J. & Chen, D. J. DNA double strand break repair via non-homologous end-joining. *Transl. Cancer Res.* **2**, 130 (2013).
- Chang, H. H. Y., Pannunzio, N. R., Adachi, N. & Lieber, M. R. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat. Rev. Mol. Cell Biol.* **18**, 495–506 (2017).
- Pecina-Slaus, N., Kafka, A., Salamon, I. & Bukovac, A. Mismatch repair pathway, genome stability and cancer. *Front. Mol. Biosci.* **7**, 122 (2020).
- Yousefzadeh, M. et al. DNA damage-how and why we age? *Elife* **10**, 1–17 (2021).
- Basu, A. K. DNA damage, mutagenesis and cancer. *Int. J. Mol. Sci.* **19**, 970 (2018).
- Piett, C. G., Pecun, T. J., Laverty, D. J. & Nagel, Z. D. Large-scale preparation of fluorescence multiplex host cell reactivation (FM-HCR) reporters. *Nat. Protoc.* **16**, 4265–4298 (2021).
- Chaim, I. A. et al. In vivo measurements of interindividual differences in DNA glycosylases and APE1 activities. *Proc. Natl. Acad. Sci. U. S. A.* **114**, E10379–E10388 (2017).
- Toprani, S. M. et al. High-throughput screening platform for nanoparticle-mediated alterations of DNA repair capacity. *ACS Nano* **15**, 4728–4746 (2021).
- Bitounis, D. et al. Printer center nanoparticles alter the DNA repair capacity of human bronchial airway epithelial cells. *NanoImpact* **25**, 100379 (2022).
- Trzeciak, A. R. et al. Age, sex, and race influence single-strand break repair capacity in a human population. *Free Radic. Biol. Med.* **45**, 1631–1641 (2008).
- McNeely, E., Mordukhovich, I., Staffa, S., Tideman, S. & Coull, B. Legacy health effects among never smokers exposed to occupational secondhand smoke. *PLoS ONE* **14**, e0215445 (2019).
- Toprani, S. M., Scheibler, C. & Nagel, Z. D. Interplay between air travel, genome integrity, and COVID-19 risk vis-a-vis flight crew. *Front. Public Health* **8**, 1–6 (2020).
- Peto, R. et al. Smoking, smoking cessation, and lung cancer in the UK since 1950: Combination of national statistics with two case-control studies. *BMJ* **321**, 323–329 (2000).
- Wen, C. C. Y. et al. Health Risks and Potential Predictors of Fatigue and Sleepiness in Airline Cabin Crew. **18**, 1–15.
- Zubac, D., Stella, A. B. & Morrison, S. A. Up in the air: Evidence of dehydration risk and long-haul flight on athletic performance. *Nutrients* **12**, 1–15 (2020).
- Moro-García, M. A., Mayo, J. C., Sainz, R. M. & Alonso-Arias, R. Influence of inflammation in the process of T lymphocyte differentiation: Proliferative, metabolic, and oxidative changes. *Front. Immunol.* **9**, 1 (2018).
- Seiler, A., Fagundes, C. P. & Christian, L. M. The impact of everyday stressors on the immune system and health. In *Stress challenges and immunity in space* 71–92 (Springer, 2019). https://doi.org/10.1007/978-3-030-16996-1_6/COVER.
- Abdel-Rahman, S. Z. & El-Zein, R. A. Evaluating the effects of genetic variants of DNA repair genes using cytogenetic mutagen sensitivity approaches. *Biomarkers* **16**, 393 (2011).
- Nagel, Z. D. et al. Towards precision prevention: Technologies for identifying healthy individuals with high risk of disease. *Mutat. Res. – Fundam. Mol. Mech. Mutagen.* **800–802**, 14–28 (2017).
- Nagel, Z. D., Chaim, I. A. & Samson, L. D. Inter-individual variation in DNA repair capacity: A need for multi-pathway functional assays to promote translational DNA repair research. *DNA Repair (Amst)* **19**, 199–213 (2014).
- Nagel, Z. D. et al. Fluorescent reporter assays provide direct, accurate, quantitative measurements of MGMT status in human cells. *PLoS ONE* **14**, e0208341 (2019).
- Qiao, Y. et al. Rapid assessment of repair of ultraviolet DNA damage with a modified host-cell reactivation assay using a luciferase reporter gene and correlation with polymorphisms of DNA repair genes in normal human lymphocytes. *Mutat. Res. – Fundam. Mol. Mech. Mutagen.* **509**, 165–174 (2002).
- Johnson, J. M. & Latimer, J. J. Analysis of DNA repair using transfection-based host cell reactivation. *Methods Mol. Biol.* **291**, 321–335 (2005).
- Cramers, P. et al. Impaired repair of ionizing radiation-induced DNA damage in Cockayne syndrome cells. *Radiat. Res.* **175**, 432–443 (2011).

47. Krokan, H. E. & Bjørås, M. Base excision repair. *Cold Spring Harb. Perspect. Biol.* **5**, 1–22 (2013).
48. Nagel, Z. D. et al. DNA repair capacity in multiple pathways predicts chemoresistance in glioblastoma multiforme. *Cancer Res.* <https://doi.org/10.1158/0008-5472.CAN-16-1151> (2017).
49. Cheong, A. & Nagel, Z. D. Human variation in DNA repair, immune function, and cancer risk. *Front. Immunol.* **13**, 899574 (2022).
50. Fieres, J. et al. The burden of overweight: Higher body mass index, but not vital exhaustion, is associated with higher DNA damage and lower DNA repair capacity. *DNA Repair (Amst.)* **114**, 103323 (2022).
51. Tyson, J. et al. Inter-individual variation in nucleotide excision repair in young adults: Effects of age, adiposity, micronutrient supplementation and genotype. *Br. J. Nutr.* **101**, 1316–1323 (2009).
52. Toprani, S. M. S. & Das, B. Radio-adaptive response of base excision repair genes and proteins in human peripheral blood mononuclear cells exposed to gamma radiation. *Mutagenesis* **30**, 663–676 (2015).
53. Vogel, U. et al. Inter-individual variation, seasonal variation and close correlation of OGG1 and ERCC1 mRNA levels in full blood from healthy volunteers. *Carcinogenesis* **23**, 1505–1509 (2002).
54. Cherednichenko, O., Pilyugina, A. & Nuraliev, S. Chronic human exposure to ionizing radiation: Individual variability of chromosomal aberration frequencies and G0 radiosensitivities. *Mutat. Res. Toxicol. Environ. Mutagen.* **873**, 503434 (2022).
55. Toprani, S. M. & Das, B. Radio-adaptive response, individual radio-sensitivity and correlation of base excision repair gene polymorphism (hOGG1, APE1, XRCC1, and LIGASE1) in human peripheral blood mononuclear cells exposed to gamma radiation. *Environ. Mol. Mutagen.* **61**, 551–559 (2020).
56. Crespo-Orta, I., Ortiz, C., Encarnación, J., Suárez, E. & Matta, J. Association between DNA repair capacity and body mass index in women. *Mutat. Res.* **826**, 111813 (2023).
57. Davis, A. J., Chen, B. P. C. & Chen, D. J. DNA-PK: A dynamic enzyme in a versatile DSB repair pathway. *DNA Repair (Amst.)* **17**, 21–29 (2014).
58. Buja, A. et al. Cancer incidence among female flight attendants: A meta-analysis of published data. *J. Womens Health (Larchmt.)* **15**, 98–105 (2006).
59. Cone, J. E., Vaughan, L. M., Huete, A. & Samuels, S. J. Reproductive health outcomes among female flight attendants: An exploratory study. *J. Occup. Environ. Med.* **40**, 210–216 (1998).
60. National Council on Radiation Protection and Measurements. *Ionizing radiation exposure of the population of the United States. Report No. 160. Council on Radiation Protection and Measurements (NCRP)*. Bethesda, MD: (2009).
61. Heylmann, D., Ponath, V., Kindler, T. & Kaina, B. Comparison of DNA repair and radiosensitivity of different blood cell populations. *Sci. Rep.* **11**, 1–13 (2021).
62. Cadilhac, P. et al. In-flight ultraviolet radiation on commercial airplanes. *Aerosp. Med. Hum. Perform.* **88**, 947–951 (2017).
63. Aw, J. J. Cosmic radiation and commercial air travel. *J. Travel Med.* **10**, 19–28 (2003).
64. Goldhagen, P. Overview of aircraft radiation exposure and recent ER-2 measurements. *Health Phys.* **79**, 526–544 (2000).
65. ICRP. International Commission on Radiological Protection (ICRP) Publication 103: Recommendations of the International Commission on Radiological Protection. *Radiat. Prot. Dosim.* **129**, 500–507 (2008).
66. Sridharan, D. M. et al. Evaluating biomarkers to model cancer risk post cosmic ray exposure. *Life Sci. Space Res.* **9**, 19–47 (2016).
67. Soren, D. C., Toprani, S. M., Jain, V., Saini, D. & Das, B. Quantitation of genome damage and transcriptional profile of DNA damage response genes in human peripheral blood mononuclear cells exposed in vitro to low doses of neutron radiation. *Int. J. Radiat. Res.* **17**, 1–14 (2019).
68. Toprani, S. M. & Das, B. Role of base excision repair genes and proteins in gamma-irradiated resting human peripheral blood mononuclear cells. *Mutagenesis* **30**, 247–261 (2015).
69. Hall, E. J. & Giaccia, A. J. *Radiobiology for the Radiologist, 7th Edition. Radiobiology for the Radiologist: Seventh Edition* (Wolters Kluwer Health Adis (ESP), 2012). <https://doi.org/10.1016/J.JROBP.2006.06.027>
70. Kelsey, C. A. et al. Radiation effects on tissues and organs. In *Radiation biology of medical imaging* (ed. Raj, E.) (Wiley, 2014). <https://doi.org/10.1002/9781118517154.CH9>
71. Ministry of the Environment Government of Japan (JCN1000012110001). Radiosensitivity of organs and tissues [MOE]. In *Booklet to Provide Basic Information Regarding Health Effects of Radiation* (2013).
72. Cheng, L. et al. Simultaneous induction of dispersed and clustered DNA lesions compromises DNA damage response in human peripheral blood lymphocytes. *PLoS ONE* **13**, e0204068 (2018).
73. Bauchinger, M. Quantification of low-level radiation exposure by conventional chromosome aberration analysis. *Mutat. Res. Genet. Toxicol.* **339**, 177–189 (1995).
74. Cologne, J. et al. Chromosome aberrations among atomic-bomb survivors exposed in utero: Updated analysis accounting for revised radiation doses and smoking. *Radiat. Environ. Biophys.* **61**, 59–72 (2022).
75. Lomax, M. E., Folkes, L. K. & O'Neill, P. Biological consequences of radiation-induced DNA damage: Relevance to radiotherapy. *Clin. Oncol. (R. Coll. Radiol.)* **25**, 578–585 (2013).
76. Komov, O. et al. Relationship between radioadaptive response and individual radiosensitivity to low doses of gamma radiation: An extended study of chromosome damage in blood lymphocytes of three donors. *Int. J. Radiat. Biol.* **94**, 54–61 (2018).
77. Yao, Y. & Dai, W. Genomic instability and cancer. *J. Carcinog. Mutagen.* **5**, 1000165 (2015).
78. Knijnenburg, T. A. et al. Genomic and molecular landscape of DNA damage repair deficiency across the cancer genome atlas. *Cell Rep.* <https://doi.org/10.1016/j.celrep.2018.03.076> (2018).
79. Kawanishi, S., Ohnishi, S., Ma, N., Hiraku, Y. & Murata, M. Crosstalk between DNA damage and inflammation in the multiple steps of carcinogenesis. *Int. J. Mol. Sci.* **18**, 1808 (2017).
80. Kay, J., Thadhani, E., Samson, L. & Engelward, B. Inflammation-induced DNA damage, mutations and cancer. *DNA Repair (Amst.)* **83**, 102673 (2019).
81. Castro, M., Carvalhais, J. & Teles, J. Irregular working hours and fatigue of cabin crew. *Work* **51**, 505–511 (2015).
82. Haus, E. L. & Smolensky, M. H. Shift work and cancer risk: Potential mechanistic roles of circadian disruption, light at night, and sleep deprivation. *Sleep Med. Rev.* **17**, 273–284 (2013).
83. Leproult, R., Holmbäck, U. & Van Cauter, E. Circadian misalignment augments markers of insulin resistance and inflammation, independently of sleep loss. *Diabetes* **63**, 1860–1869 (2014).
84. Boudjeltia, K. Z. et al. Sleep restriction increases white blood cells, mainly neutrophil count, in young healthy men: A pilot study. *Vasc. Health Risk Manag.* **4**, 1467 (2008).
85. Liu, H., Wang, G., Luan, G. & Liu, Q. Effects of sleep and sleep deprivation on blood cell count and hemostasis parameters in healthy humans. *J. Thromb. Thrombolysis* **28**, 46–49 (2009).
86. Song, N. et al. Whole body vibration triggers a change in the mutual shaping state of intestinal microbiota and body's immunity. *Front. Bioeng. Biotechnol.* **7**, 377 (2019).
87. Silva, R. et al. Occupational cosmic radiation exposure in Portuguese airline pilots: Study of a possible correlation with oxidative biological markers. *Radiat. Environ. Biophys.* **52**, 211–220 (2013).
88. Ohkoshi, H. et al. The influence of transmeridian flight on human circulating lymphocytes. *Aviat. Space Environ. Med.* **62**, 14–18 (1991).
89. Chiorcea-Paquim, A. M. 8-oxoguanine and 8-oxodeoxyguanosine biomarkers of oxidative DNA damage: A review on HPLC-ECD determination. *Molecules* **27**, 1620 (2022).

90. Cordero, C. et al. Contributing factors to the oxidation-induced mutational landscape in human cells. *Nat. Commun.* **15**, 1–18 (2024).
91. Ba, X. & Boldogh, I. 8-Oxoguanine DNA glycosylase 1: Beyond repair of the oxidatively modified base lesions. *Redox Biol.* **14**, 669 (2018).
92. Karahalil, B., Bohr, V. A. & Wilson, D. M. Impact of DNA polymorphisms in key DNA base excision repair proteins on cancer risk. *Hum. Exp. Toxicol.* <https://doi.org/10.1177/0960327112444476> (2012).
93. Cavallo, D. et al. Evaluation of DNA damage in flight personnel by Comet assay. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **516**, 148–152 (2002).
94. Minorette, P., Riera, M. L., Sáez, A. S., Serrano, M. G. & Martín, Á. G. Increased peripheral blood DNA damage and elevated serum levels of melanoma inhibitory activity protein: Clues to excess skin cancer risk in airline pilots?. *Cureus* **15**, e51077 (2023).
95. Heimers, A. Chromosome aberration analysis in Concorde pilots. *Mutat. Res.* **467**, 169–176 (2000).
96. Romano, E., Ferrucci, L., Nicolai, F., Derme, V. & De Stefano, G. F. Increase of chromosomal aberrations induced by ionising radiation in peripheral blood lymphocytes of civil aviation pilots and crew members. *Mutat. Res.* **377**, 89–93 (1997).
97. Nicholas, J. S. et al. Stable chromosome aberrations and ionizing radiation in airline pilots. *Aviat. Space Environ. Med.* **74**, 953–956 (2003).
98. Yong, L. C. et al. Increased frequency of chromosome translocations in airline pilots with long-term flying experience. *Occup. Environ. Med.* **66**, 56–62 (2009).
99. Grajewski, B. et al. Chromosome translocations and cosmic radiation dose in male U.S. commercial airline pilots. *Aerosp. Med. Hum. Perform.* **89**, 616–625 (2018).
100. Paz-Elizur, T. et al. DNA repair biomarker for lung cancer risk and its correlation with airway cells gene expression. *JNCI Cancer Spectr.* **4**, pkz067 (2020).
101. Machella, N. et al. Double-strand breaks repair in lymphoblastoid cell lines from sisters discordant for breast cancer from the New York site of the BCFR. *Carcinogenesis* **29**, 1367–1372 (2008).
102. Wei, Q., Matanoski, G. M., Farmer, E. R., Hedayati, M. A. & Grossman, L. Dna repair and susceptibility to basal cell carcinoma: A case-control study. *Am. J. Epidemiol.* <https://doi.org/10.1093/oxfordjournals.aje.a117297> (1994).
103. Wei, Q., Cheng, L., Hong, W. K. & Spitz, M. R. Reduced DNA repair capacity in lung cancer patients. *Cancer Res.* **56**, 4103–4107 (1996).
104. Paz-Elizur, T. DNA repair activity for oxidative damage and risk of lung cancer. *CancerSpectr. Knowl. Environ.* <https://doi.org/10.1093/jnci/djg033> (2003).
105. Andersen, M. H. G. et al. Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. *Mutagenesis* **33**, 105–115 (2018).
106. Vlachogiannis, N. I. et al. Chronological age and DNA damage accumulation in blood mononuclear cells: A linear association in healthy humans after 50 years of age. *Int. J. Mol. Sci.* **24**, 7148 (2023).
107. Matt, K. & Bergemann, J. Ex vivo analysis of DNA repair capacity of human peripheral blood mononuclear cells by a modified host cell reactivation assay. *Bio-Protoc.* **9**, e3325 (2019).
108. Bankoglu, E. E. et al. Effect of cryopreservation on DNA damage and DNA repair activity in human blood samples in the comet assay. *Arch. Toxicol.* **95**, 1831 (2021).
109. Ngo, L. P. et al. CometChip analysis of human primary lymphocytes enables quantification of inter-individual differences in the kinetics of repair of oxidative DNA damage. *Free Radic. Biol. Med.* **174**, 89–99 (2021).
110. Zwingmann, I. et al. Oxidative DNA damage and cytogenetic effects in flight engineers exposed to cosmic radiation - PubMed. *Environ. Mol. Mutagen.* **32**, 121–129 (1998).
111. Yoo, S. S. et al. Mitigating the risk of radiation-induced cancers: Limitations and paradigms in drug development. *J. Radiol. Prot.* **34**, R25 (2014).
112. StemCell Technologies. SepMate PBMC isolation. 1–24 https://cdn.stemcell.com/media/files/pis/10000003788-PIS_09.pdf (2023).
113. Ge, J. et al. Cometchip: A high-throughput 96-well platform for measuring DNA damage in microarrayed human cells. *J. Vis. Exp.* <https://doi.org/10.3791/50607> (2014).
114. Lorenzo, Y., Costa, S., Collins, A. R. & Azqueta, A. The comet assay, DNA damage, DNA repair and cytotoxicity: Hedgehogs are not always dead. *Mutagenesis* **28**, 427–432 (2013).
115. GraphPad Software, I. GraphPad Prism 9 Curve Fitting Guide - Equation: One phase decay. https://www.graphpad.com/guides/prism/latest/curve-fitting/reg_exponential_decay_1phase.htm.
116. Kiziltepe, T. et al. Delineation of the chemical pathways underlying nitric oxide-induced homologous recombination in mammalian cells. *Chem. Biol.* **12**, 357–369 (2005).
117. R Core Team. R: A language and environment for statistical computing. *R Found Stat Comput Vienna* (2021).
118. Taiyun Wei, A. & Simko, V. R package 'corrplot': Visualization of a Correlation Matrix (version 0.92). (2021).
119. Anusha, P. V., Anuradha, C., Chandra Murty, P. S. R. & Kiran, C. S. Detecting outliers in high dimensional data sets using Z-score methodology. *Int. J. Innov. Technol. Explor. Eng.* **9**, 48–53 (2019).

Acknowledgements

We acknowledge Daniel Dawes (Brigham Women Hospital, Boston, MA) for his expertise in performing the differential blood cell counts. We acknowledge Dr. Bevin Engelward (Massachusetts Institute of Technology, Cambridge, MA) for providing guidance and PDMS stamp needed for generating CometChips, and Christopher Scheibler (Senior Flight Surgeon) for evaluating the training of phlebotomists during their recruitment. Furthermore, we extend our appreciation to union members, flight attendant association, all the phlebotomists, participants, and personnel involved for this study.

Author contributions

SMT: Contributed to conception of the study, recruited and trained phlebotomists, coordinated blood sample collection and processing, contributed to development of human subjects protocol submitted to IRB, conducted experiments, analyzed and interpreted data, and drafted the manuscript. IM: Led development of human subject's protocol submitted to IRB, recruited participants, and conceived, recruited phlebotomists, coordinated with blood sample collection, and reviewed the manuscript. EM and ZDN: Contributed to conception of the study, interpreted data, directed the overall research project, and drafted and revised the manuscript.

Funding

This work was supported by Harvard Chan NIEHS Center pilot project grant (P30ES000002). Sneha M Toprani and Zachary D Nagel were also supported by U01ES029520. Zachary D. Nagel is also supported by grants from the National Institutes of Health (R37CA248565). Irina Mordukhovich and Eileen McNeely were supported by FAMRI (Flight Attendant Medical Research Institute).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-98934-9>.

Correspondence and requests for materials should be addressed to Z.D.N.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025