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A pooled analysis of molecular epidemiological studies on modulation of DNA repair by host factors

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ABSTRACT

Levels of DNA damage represent the dynamics between damage formation and removal. Therefore, to better interpret human biomonitoring studies with DNA damage endpoints, an individual's ability to recognize and properly remove DNA damage should be characterized. Relatively few studies have included DNA repair as a biomarker and therefore, assembling and analyzing a pooled database of studies with data on base excision repair (BER) was one of the goals of hCOMET (EU-COST CA15132). A group of approximately 1911 individuals, was gathered from 8 laboratories which run population studies with the comet-based *in vitro* DNA repair assay. BER incision activity data were normalized and subsequently correlated with various host factors. BER was found to be significantly higher in women. Although it is generally accepted that age is inversely related to DNA repair, no overall effect of age was found, but sex differences were most pronounced in the oldest quartile (>61 years). No

Abbreviations: FaPy-G, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-oxo-G, 7,8-dihydro-8-oxo-guanine; BER, base excision repair; BMI, body mass index; Fpg, formamidopyrimidine DNA glycosylase; SSB, single-strand break.

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effect of smoking or occupational exposures was found. A body mass index (BMI) above 25 kg/m^2 was related to higher levels of BER. However, when BMI exceeded 35 kg/m^2, repair incision activity was significantly lower. Finally, higher BER incision activity was related to lower levels of DNA damage detected by the comet assay in combination with formamidopyrimidine DNA glycosylase (Fpg), which is in line with the fact that oxidatively damaged DNA is repaired by BER. These data indicate that BER plays a role in modulating the steady-state level of DNA damage that is detected in molecular epidemiological studies and should therefore be considered as a parallel endpoint in future studies.

1. Introduction

Our genome is constantly exposed to DNA-damaging agents and therefore its maintenance is critical for the preservation of genetic information. As a response to DNA damage, a network of events collectively termed as the DNA damage response is activated, including activation of cell-cycle checkpoints, DNA damage recognition and subsequent DNA repair [1]. The DNA repair pathways are responsible for removing the damage in a substrate-dependent manner and the type of DNA repair pathway that is activated depends largely on the type of DNA damage and stage of the cell cycle. The major DNA repair pathway that corrects non-bulky damage of DNA bases caused by alkylation, oxidation, uracil incorporation, deamination and depurination, as well as repairing single-strand breaks (SSBs), is base excision repair (BER) [2,3]. BER is a multistep process, initiated by a damage-specific DNA glycosylase that recognizes the damaged DNA base and cleaves the N-glycosylic bond that links a DNA base to the sugar-phosphate backbone. The resulting baseless sugar (also called abasic site, apurinic/apyrimidinic site or AP site) is further processed by an AP endonuclease that cleaves the phosphodiester bond 5` to the AP site, thus generating a SSB (for review see [4,5]). The fact that the DNA repair machinery generates transient DNA strand breaks makes it possible to detect DNA repair incision activity with single cell gel-electrophoresis, also known as the comet assay. Indeed, a comet-based *in vitro* DNA repair assay was first developed to detect the incision activity at oxidatively damaged DNA. This method has been successfully used to monitor DNA repair of oxidized DNA lesions in humans, but unfortunately the number of studies is low [6–9].

The most vulnerable base to oxidation is guanine, due to its low reduction potential and high nucleophilicity [10], leading to the formation of 7,8-dihydro-8-oxo-guanine (8-oxo-G) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FaPy-G) [5]. These lesions are highly mutagenic and if not repaired, the damaged base can pair with adenine during DNA replication, causing predominantly G:C to T:A transversions [11–13]. Since 8-oxo-G is a major type of DNA damage occurring after oxidative stress, it is widely accepted that 8-oxo-G is a potential biomarker for measuring oxidative DNA damage in human biomonitoring studies. However, the amount of 8-oxo-G that is measured at any given time point is the net result of both DNA damage formation and removal. In other words, a low level of 8-oxo-G could be the result of low levels of exposure to DNA damaging compounds or highly efficient removal/repair of the lesions, or a combination of both. *Vice versa*, high levels of damage could mean high levels of exposure or low DNA repair efficiency. Therefore, for the correct interpretation of human biomonitoring studies and to avoid miss-classification, it is important to understand the underlying mechanisms that lead to the levels of a specific type of DNA damage as detected in human samples. To improve the application of DNA repair measurements in future biomonitoring studies, we need to consider the extent in which DNA repair incision activity can vary between individuals and what are the underlying reasons for this variation. Moreover, it is also necessary to understand whether there is a direct relationship between DNA repair and the level of DNA damage in healthy individuals.

In the hCOMET COST action (www.hcomet.eu), we therefore pooled data from 8 molecular epidemiological studies and linked DNA repair incision activity data (assessed with the comet-based *in vitro* DNA repair assay) to personal data, including sex, smoking behaviour, occupational exposures, age and BMI. Additionally, we investigated whether high DNA repair incision activity is related to the amount of DNA damage (specifically 8-oxo-G) in healthy volunteers.

2. Materials and methods

2.1. The hCOMET DNA repair database

Eight molecular epidemiological studies contributed data referring to 1911 subjects within the framework of the hCOMET COST action CA15132. Data included in the database were published between 2008 and 2014 [14–18, and unpublished data]; all laboratories used the comet assay to assess DNA damage and BER incision activity in peripheral blood cells in human populations. All studies included in the analysis described the epidemiological design, had a control group, and reported an adequate description of the protocol(s) used. Whenever available in the original set of data, detailed information was collected on demographic parameters, lifestyle, occupational exposure, smoking habit, diet, genetic profile, and diagnoses of chronic diseases. The large heterogeneity among the laboratories contributing data, in terms of quality and quantity of information collected, did not allow fine-tuning in the analysis of parameters, apart from age, sex, BMI, smoking and occupational exposure (mainly to asbestos, stone wool, glass fibres *via* inhalation) (Table 1A). For smoking habits subjects were classified as non-smokers, former smokers, and current smokers.

Data gathering was coordinated by the IRCCS San Raffaele Pisana, Rome, Italy, and the Institute for Medical Research and Occupational Health, Zagreb, Croatia. The pooled analysis of data was approved by the Ethics Committee of the IRCCS San Raffaele Pisana, Rome, Italy, *i.e.*, the centre coordinating data collection and supervising the statistical analysis of data. Each study had already ethical approval from local Ethical Committees for the collection and analysis of individual coded data, and all General Data Protection Regulations (GDPR) were respected.

2.2. Assessment of base excision repair by the comet assay

An extensive questionnaire collecting technical details of protocols used by participating laboratories was developed by the coordinating centre. All laboratories used their own protocols, which were based on the general protocol that was recently published [19]. Laboratory specific conditions can be found in Table 1S. Samples were collected according to each laboratory ‘s own protocol (all used venepuncture) and protein extracts were prepared from isolated peripheral blood mononuclear cells according to published protocols [19]. Incubations were standardized within a laboratory by using either a fixed amount of cells when preparing the extract, or standardizing the protein concentration of the extract. DNA nucleoids containing 8-oxo-G (induced by the photosensitizer Ro 19-8022 plus light) were incubated with protein extracts containing DNA repair enzymes. These enzymes, as a part of the repair process, induce transient breaks at the site of the lesions in the substrate that were subsequently measured using the alkaline comet assay protocol. The capacity of the cell extract to carry out the incisions, considered to be the rate-limiting step of the repair process, is taken as an indicator of the DNA repair incision activity of the cells. All studies in

the current pooled analysis used the photosensitiser Ro 19-8022 plus visible light variant of the comet-based *in vitro* DNA repair assay [19].

2.3. Comet assay for strand breaks and Fpg sensitive sites

Strand breaks were measured by the alkaline comet assay and formamidopyrimidine DNA glycosylase (*Fpg*)-sensitive sites (which includes 8-oxo-G and other oxidized DNA bases such as FapyA and FapyG) [20] were measured with the enzyme-modified alkaline comet assay. The protocols were essentially the same for all labs [21], with only minor deviations due to lab-specific conditions. In brief, cells were mixed with low melting point agarose and added to pre-coated glass slides or GelBond film. Samples were then placed in lysis solution at 4 °C with 1 % Triton X-100 (with or without 10 % DMSO) at 4 °C for at least 1 h. After lysis, slides were washed with an enzyme buffer [usually 40 mM HEPES, 0.5 mM Na_2EDTA, 0.1 M KCl and 0.2 mg/mL BSA, pH 7.6], followed by incubation at 37 °C for 30 min - 1 h in fresh enzyme buffer with or without *Fpg*. After unwinding (40 min, 4 °C) in electrophoresis solution (0.3 M NaOH, 1 mM EDTA-Na_2, pH $\geq$ 13.2), electrophoresis was performed at ~1 V/cm for approximately 20 min. Samples were neutralised and fixed in ethanol. Each lab used their own staining solution, and subsequently the gels were examined by fluorescence microscopy and at least 100 comets per sample were analysed. DNA damage was defined as %Tail DNA, *i.e.* the fluorescence intensity in the tail relative to the total intensity of the comet or by visual scoring. The net increase in DNA damage measured with Fpg (*i.e.* subtracting DNA damage obtained after buffer incubation from that obtained after Fpg incubation) is denoted as Fpg-sensitive sites.

2.4. Statistical analysis

All data were normalized towards the individual study means. So for each study, each individual value of DNA repair was divided by the mean DNA repair value of that particular study, *i.e.*:

$$Individual\ value\ in\ study\ X / Mean\ of\ study\ X$$

As a result, all studies will have a mean value of 1, whereas the coefficient of variation (CV, *i.e.*, standard deviation/average remains the same for each study. Some parameters were analysed after categorization: for BMI various categories were considered: i) first comparing overweight (BMI > 25 kg/m^2) *versus* normal weight (BMI = 15−25 kg/m^2); and ii) comparing various arbitrary weight classes – low weight (BMI = 15–19.9 kg/m^2), normal weight (BMI = 20–24.9 kg/m^2), overweight (BMI = 25–29.9 kg/m^2), class 1 obesity (BMI = 30−34.9 kg/m^2), class 2 obesity (BMI = 35–39.9 kg/m^2) and class 3 obesity (BMI > 40 kg/m^2). Age groups were made according to quartiles (Q1: ≤27 years, Q2: 28–41 years, Q3: 42–61 years, Q4: >61 years). All data were pooled and analysed by SPSS 13.0 statistical software using parametric tests, considering differences with $P < 0.05$ as statistically significant. Demographic data are presented as mean and standard deviation or range. All other data are presented as mean with standard error.

3. Results

3.1. Demographics of the study population

BER incision activity values were available for 1911 subjects of which 1005 (53 %) were female and 693 (36 %) were male; sex was not known for the remaining 213 subjects (11 %). The average age of the study population was 43.2 years, ranging from 15 to 88 years (age was available for 1536 subjects which was 81 % of the total study population). Although males were significantly older (45.3 years) than females (42.4 years, $P = 0.002$), the average difference of 3 years was not expected to affect DNA repair incision activity. Nonetheless, analyses were additionally performed in groups according to age quartiles. Most of the study-subjects were non-smokers (n = 957) or former smokers (n = 111). Three hundred and sixty subjects were active smokers at the time of sampling. Exposure data other than smoking were too diverse to make groups according to the types of exposure and were therefore grouped as exposed yes/no. Occupational exposure to potential genotoxic agents was reported by 242 subjects, including inhalation exposure to fibres such as asbestos, stone wool and glass fibre (13.4 %). The number of

Table 1
Overall description of the study population (Panel A) and summary of individual studies contributing data to the pooled analysis (panel B).

A

Descriptor	Average ± SD [Range] or N (%)
Age (years)	43.4 ± 17.5 [15−88]
Sex (male/female/unknown)	693/1005/213 (36/53/11 %)
BMI (kg/m^2)	25.1 ± 4.5 [15−56]
Smoking habit	
Non-smoker	957 (50.1 %)
Former smokers	111 (5.8 %)
Current smokers	360 (18.8 %)
Unknown	483 (25.3 %)
Occupational exposure	
Non-exposed	1387 (72.6 %)
Exposed	242 (12.7 %)
Unknown	282 (14.8 %)

B

Study number	Lab ID	N	Age Average ± SD	Sex M/F	BMI Kg/m^2	Repair (a.u.)	Type of scoring	reference*
1	EU6	341	46 ± 15	180/161	25 ± 4	43 ± 44	Visual score	[14]
2	EU22-5	156	NR	156/0	NR	78 ± 44	%Tail DNA	[16]
3	EU22-4	213	NR	NR	NR	47 ± 16	%Tail DNA	[15]
4	EU22-1	200	30 ± 6	0/200	24 ± 5	2.2 ± 2.2	%Tail DNA	[22]
5	EU31-2	388	47 ± 12	224/164	26 ± 4	114 ± 47	Visual score	[17]
6	EU31-3	319	45 ± 22	122/197	25 ± 5	97 ± 47	Visual score	[23]
7	EU31-4	273	41 ± 19	0/273	24 ± 4	108 ± 42	Visual score	[24]
8	EU33-4	21	57 ± 18	11/10	25 ± 5	16 ± 5	%Tail DNA	[18] **

NR = Not Reported.

* Please note that the data that were provided to the hComet database included unpublished data (reference describes the original study from which the data were obtained). Therefore, the number of data points in the current analysis may differ from the originally published data.

** Study subjects suffered from chronic renal failure.

subjects for which all these data were available was 1228, which is 68.4 % of the total population (Table 1A and B)

3.2. The effect of age and sex on BER incision activity

Relative BER incision activity was significantly higher in females when compared to males (Fig. 1A; 1.027 ± 0.023 *vs*. 0.955 ± 0.023, P = 0.029). Four laboratories provided data of both males and females. Three out of those 4 laboratories confirmed the higher DNA repair incision activity in females when compared to males, when analysed separately (Fig. 1S). There was no overall effect of age, but DNA repair capacity tended to increase with age in women, whereas in men BER decreased with age. As a result, stratified analysis on basis of age groups indicated that the sex difference was strongest in the oldest subjects (>61 years) (Fig. 1B). For instance, in the youngest quartile (≤27 years) females and males did not differ in repair incision activity (1.008 ± 0.035 *vs*. 0.988 ± 0.076, P = 0.797), but in the oldest group, a statistically significant 17 % difference was found with women having a relative repair capacity of 1.068 ± 0.039 and men 0.889 ± 0.050 (P = 0.008).

3.3. The influence of smoking habits and occupational exposures on BER incision activity

Exposure to genotoxic agents will damage DNA, which may subsequently induce BER incision activity. Therefore, exposure may contribute at least in part to the variation in BER observed in the current study population. Information was available about smoking behaviour and the presence of occupational exposures to various types of genotoxic agents, but predominantly exposure to particles and fibres. Subjects who reported to be smoker at the time of sample collection had a relative BER incision activity of 0.986 ± 0.027, whereas non-smokers had a relative BER incision activity of 1.005 ± 0.022 (P > 0.05). Also repair incision activity in former smokers (0.957 ± 0.098) did not significantly differ from non-smokers or active smokers. Occupational exposure to potential genotoxic substances did not affect repair incision activity (P = 0.862). These findings were not altered after stratification for age or sex, or after combining the occupational exposures with smoking status.

3.4. Differences in BER incision activity between various weight classes

Obesity is associated with increased oxidative stress [22,23] that can lead to DNA damage that is typically repaired by BER. In the current study, no overall linear relationship was found between BMI and repair incision activity. However, after setting groups according to weight classes, BER was significantly higher in overweight subjects (25 ≤ BMI<30) when compared to normal weight individuals (BMI < 25) (Fig. 2). Interestingly, in the groups with (severe) obesity (BMI ≥ 35), DNA repair incision activity is significantly lower, when compared to obese individuals (BMI 30−34 kg/m^2, P = 0.005).

3.5. Overall analysis of host factors affecting BER incision activity

An overall regression analysis was performed on all host factors. Since the relationship between BMI and BER was a non-linear relationship - higher levels of BER in overweight subjects, but significantly lower BER incision activity in obese individuals (BMI > 35) - the overall regression analysis was restricted to individuals with a BMI < 35. In this analysis, only sex was a significant predictor of BER incision activity (P = 0.007), whereas all other host factors did not reach statistical significance (Table 2).

3.6. Correlation between BER incision activity and levels of DNA damage

Since BER specifically removes oxidatively damaged lesions from the DNA (including 8-oxo-G), which can be detected by the Fpg-modified comet assay, it was expected that high BER incision activity would be

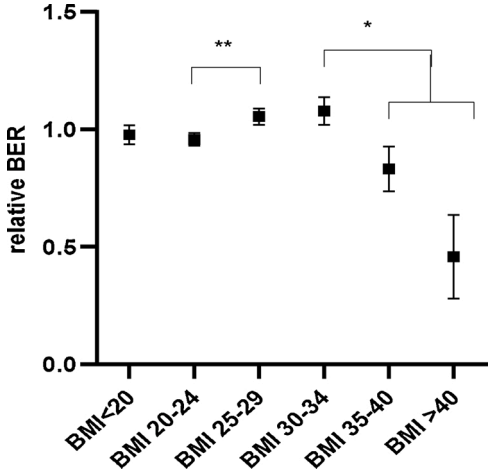

Fig. 2. Relationship between various classes of BMI and relative BER activity. (*P = 0.014, **P = 0.005). Number of subjects in each category: N = 160, 620, 445, 147, 31 and 5, respectively for groups with increasing BMI.

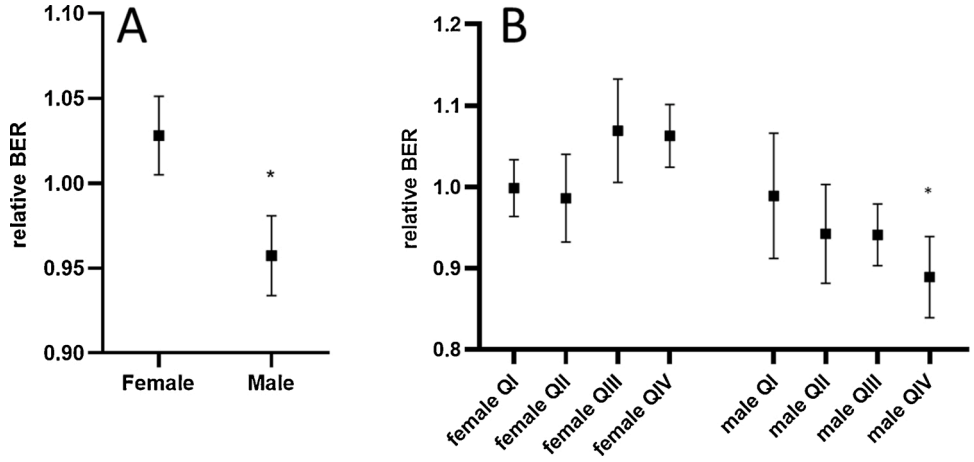

Fig. 1. Overall difference between males (N = 672) and females (N = 910) in relative BER incision activity: P = 0.029 (**A**), and the combined effect of sex and age on relative BER capacity (**B**). (Age groups QI: ≤27 years, QII: 28-41 years, QIII: 42-61 years, QIV: >61 years). Difference between males and females in the oldest quartile was statistically significant (*P = 0.008).

Table 2
Multiple regression analysis with all (standardized) host factors as independent and repair activity as dependent variable, including individuals with a BMI < 35.

Host variable	Beta ± SE	P-value
Constant	0.929 ± 0.119	0.000
BMI (kg/m^2)	0.005 ± 0.005	0.362
Exposures		
Smoking (0/1/2)	0.009 ± 0.022	0.685
Occupational exposure	−0.006 ± 0.045	0.899
Sex (female/male)	−0.104 ± 0.039	0.007
Age (years)	−0.002 ± 0.018	0.903

N = 1227.

linked to lower levels of DNA damage. Indeed, an inverse correlation was found between oxidative DNA damage and relative BER incision activity (R = -0.086, P = 0.0007) (Fig. 3A). After grouping individuals on basis of DNA repair as higher or lower than 1.00, a highly significant difference in normalized DNA damage as detected by the Fpg-comet assay was found (Fig. 3B, P < 0.001).

Background levels of DNA strand breaks may in part be related to transient strand breaks induced by the BER machinery, because the formation and removal of DNA damage is a continuous process. We therefore also correlated BER incision activity with the alkaline comet assay data (representing strand breaks), but no significant relationship was found. Subjects with a low normalized repair rate (<1.00) did not differ from subjects with a relatively high repair rate (>1.00) regarding the amount of strand breaks (normalized data: 1.014 ± 0.025 *vs.* 0.991 ± 0.027, respectively, P = 0.524) (Fig. 3C).

4. Discussion

DNA damage plays an important role in the initiation of carcinogenesis and therefore it is often used as an endpoint in molecular epidemiological studies. Indeed, in the hCOMET COST Action (CA15132), we recently showed in a prospective study that high levels of DNA damage determined by the comet assay reflect an increased incidence of mortality and cancer later in life [24]. Also impaired DNA repair is associated with various diseases including cancer [25,26], neurological disorders [27], immunodeficiency disorders [28] and several heritable syndromes [29]. In other words, an individual with a relatively low exposure to genotoxic agents may still be at risk, if that same individual also has a low level of DNA repair and thus can accumulate damage to higher levels. Therefore, an individual's ability to properly remove DNA damage could be assessed in molecular epidemiological studies to complement the interpretation of DNA damage endpoints and the individual's risk to develop pathologies related to genetic damage. In hCOMET, we analysed a pooled database of ~1900 individuals to discover the relationship between DNA repair and different host parameters to understand potential sources of inter-individual variation.

BER can be measured by a modified comet assay and we pooled data from 8 studies that specifically analysed the capacity for repair of 8-oxo-G (an important marker for oxidative stress-induced DNA damage). Overall, we showed that women have relatively higher levels of BER compared to men, especially at older ages. There was no overall age effect on repair incision activity, and neither did our analysis show an effect of smoking habits on the level of DNA repair. We did however observe a non-linear impact of BMI on DNA repair, with increased BER in overweight subjects, but lower levels of repair in obese subjects with a BMI > 35 kg/m^2.

The higher levels of BER in females is in line with the incidence of and mortality from several diseases, including the sex-specific disparities in cancer [30,31]. Recent studies have shown that male cancer incidence is about 20 % and mortality about 40 % higher compared to females [32]. Sex differences in cancer incidence may be at least partly associated with regulation of repair or the DNA damage response at the molecular level. There is evidence that females are more resistant to the loss of function of tumour suppressor protein p53 [33]. Moreover, MDM2, which is the main regulator of p53, is strongly affected by oestrogen signalling [34]. Interestingly, oestrogen and oestrogen metabolites have been shown to influence the generation of reactive oxygen species (ROS) [35], that can subsequently promote DNA base oxidation damage [36]. This generation of oxidative stress or DNA damage may be a stimulus for activation of BER. However, this is not in line with our observation that the sex disparity is strongest at older ages, because oestrogen levels decrease in post-menopausal women. After menopause, oestrogen production is strongly reduced (~60 %), but the production of progesterone is lost almost completely. Therefore, the balance between these hormones changes with increasing age. Our data warrant the further analysis of sex differences in DNA repair taking age into account, because of the potential modulation of DNA repair by sex-related hormones.

It is widely accepted that DNA damage and mutations accumulate with age and that this phenomenon is associated with reduced DNA repair [37]. In our study no statistically significant overall effect of age was found; however, DNA repair capacity tended to increase with age in females, while in males BER decreased with age as expected from the literature. In our study BER incision activity was measured by a modified comet assay, which mainly measures DNA incisions at the site of damage [19]. A study by Mikkelsen et al. in mice showed that the activity of Ogg1 and Neil1 remained constant with age [38]. Ogg1 recognises and excises 8-oxo-G from DNA. Neil1 mainly recognizes formamidopyrimidine derivates of guanine and adenine, 5-hydroxyuracil, and thymine glycol [39], but it can also recognize 8-oxo-G when it is located near the 3′-end of single strand breaks and DNA bubble structures [38]. Still, the level of 8-oxo-G increased with age in liver and lung in these mice. Although *in vitro*, OGG1 activity may decline over time when culturing cells [40], we speculate that the accumulation of

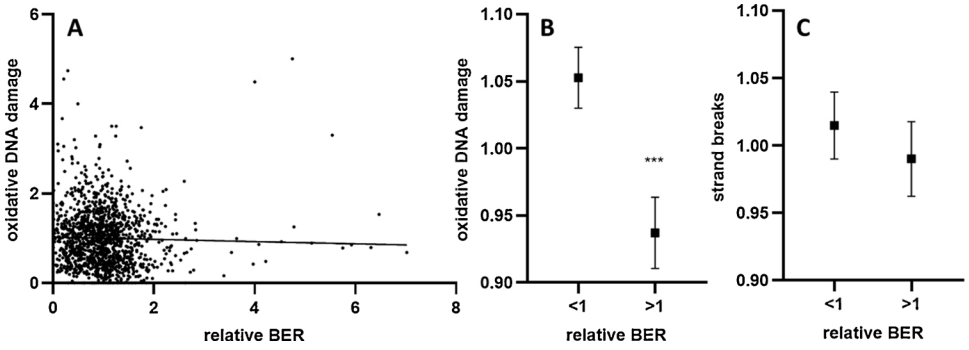

Fig. 3. Correlation between DNA incision activity and DNA oxidation damage in white blood cells of volunteers (n = 1390) (**A**). The group of subjects with relative DNA repair activity ≥1.00 showed significantly lower levels of DNA oxidation damage when compared to the group with BER < 1.00 (P < 0.001) (**B**), but no significant effect of DNA repair was found on DNA strand breaks assessed by the comet assay (P = 0.524) (**C**).

8-oxo-G with age is not a direct consequence of decreased enzyme activity but probably involves other pathways (*e.g.*, presence of antioxidants) that were not assessed in parallel in our study. Indeed, there is evidence that in some organs (*e.g.* intestine, spleen, testis) DNA repair activity does not change with age [41]. There is compelling evidence that aging-associated accumulation of oxidatively damaged DNA predominantly occurs in organs with limited cell proliferation. Indeed, a study by Langie et al. in aging mice showed that BER incision activity was significantly reduced in brain tissue and was associated with increased 8-oxo-G levels [42]. Therefore, repair measured in our surrogate tissue (*i.e.*, white blood cells), a tissue with high levels of proliferation, may not necessarily reflect the repair in all types of tissue. In general, various studies have shown a correlation between surrogate and target tissue [8], though the number of studies is limited and therefore, this aspect deserves further attention.

It was expected that exposure to oxidative stressors and oxidative DNA damaging agents might induce BER to cope with the increased burden. We had data available on smoking behaviour, and cigarette smoke is thought to cause oxidative stress and considered to be an important risk factor for neurodevelopmental and neurodegenerative disorders, respiratory diseases as well as cancer. In previous studies, cigarette smoke exposure reduced both nucleotide excision repair and BER in lung tissue of mice [43]. The role of smoking as a source of DNA oxidation damage in human lung is, however, not straightforward; Godschalk et al. showed that the oxidative stress-related DNA damage was not increased in lungs of smokers [44]. Similarly, in the present study we did not observe any significant association between BER and smoking habits. This may again also be related to the types of tissues that we used for our study; we measured BER in white blood cells whereas some studies that showed decreased BER after cigarette smoke exposure measured BER in target tissues, such as lung [43]. Nonetheless, our data are in line with previous findings that could not find an effect of smoking on DNA damage levels in peripheral blood [45].

We observed a non-linear relationship between BMI and BER incision activity with higher levels of BER in overweight subjects, but significantly lower BER incision activity in obese individuals (BMI $>$ 35). Obesity has been defined as an important risk factor for many diseases as such as cardiovascular diseases, diabetes, and cancer [46]. Previous studies already indicated that there is a link between overweight and DNA repair pathways [23] but overall, the knowledge concerning the impact of increased body weight and DNA damage on DNA repair is limited and warrants further research. The relationship between BMI and nucleotide excision repair has been previously studied with a modified comet assay, clearly showing an inverse association between BMI and NER in lymphocytes of young adults [47]. Here, we show that BER, too, differs in people with various levels of obesity. Initially, BER incision activity is increased, presumably as a reaction to increased levels of oxidative stress that accompany obesity. In mice, Ogg1, Neil1 and Nth1 expression was induced in reaction to a high fat diet [48]. However, in severely obese subjects (BMI $>$ 35 kg/m^2), BER activity was significantly lower. It can be speculated that in this case even higher levels of oxidative stress reduce BER incision activity [49], *e.g.* due to oxidation of protein thiols and loss of enzyme activity [50,51].

Alternatively, it is also possible that the causal relationship is the reverse; *i.e.*, that lower repair leads to increased risk of obesity. Indeed, Ogg1 knockout mice (*Ogg1*−/−) develop signs of metabolic syndrome, including increased adiposity, fatty liver, and elevated triglycerides. This is also in line with a study by Vartanian et al., where *Neil1*-deficient mice developed obesity and fatty liver disease in the absence of exogenous oxidative stress [52]. Few human studies support the role of DNA repair genes in metabolic dysfunction; the genetic variant *OGG1* Ser326Cys was reported to be associated with Type 2 diabetes [53,54]. It should be noted that this variant is present in 40–60 % and 25–40 % of the Asian and Caucasian populations, respectively [55]. These data indicate that deficient DNA repair, either due to genetic variations, epigenetic changes or other gene-environment interactions, can increase the risk of developing obesity.

It was expected that high levels of DNA repair would lead to lower levels of oxidation lesions as assessed by the Fpg-modified comet assay. Indeed, we observed a negative correlation between BER and DNA oxidation damage. Strand breaks, measured by alkaline comet assay, did not show a correlation. This suggests that our BER assay is specific for the repair of DNA oxidation damage. 8-oxo-G is accepted as a biomarker for DNA oxidation damage in biomonitoring studies, and our results indicate that BER measured by the comet-based *in vitro* DNA repair assay is specific to this type of DNA damage.

Overall, we have shown that host characteristics can determine part of the inter-individual variation in BER incision activity and that relatively low levels of repair coincide with higher levels of DNA oxidation damage. The strongest and most consistent effects were observed for sex and BMI, which deserve further attention to elucidate underlying mechanisms. These data indicate that BER measured by comet-base *in vitro* DNA repair assay should be taken into account as an endpoint in future genetic epidemiology biomonitoring studies.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mrgentox.2022.503447.

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