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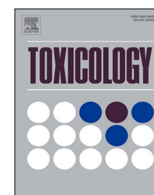

Methylmercury chronic exposure affects the expression of DNA single-strand break repair genes, induces oxidative stress, and chromosomal abnormalities in young dyslipidemic *APOE* knockout mice

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ABSTRACT

Mercury (Hg) is one of the most toxic environmental pollutants, especially when methylated, forming methylmercury (MeHg). MeHg affects DNA repair, increases oxidative stress, and predisposes to cancer. MeHg neurotoxicity is well-known, but recently MeHg-associated cardiovascular effects were recognized. This study evaluated circulating lipids, oxidative stress, and genotoxicity after MeHg-chronic exposure (20 mg/L in drinking water) in C57BL/6J wild-type and *APOE* knockout (ko) mice, the latter, being spontaneously dyslipidemic. Experimental mice were assigned to four groups: non-intoxicated and MeHg-intoxicated wild-type mice and non-intoxicated and MeHg-intoxicated *APOE* ko mice. Plasma levels of triglycerides, total cholesterol (TC), HDL, and LDL were analyzed. Liver lipid peroxidation and splenic gene expression of xeroderma pigmentosum complementation groups A, C, D, and G (*XPA*, *XPC*, *XPD*, and *XPG*), X-ray repair cross-complementing protein 1 (*XRCC1*), and telomerase reverse transcriptase (*TERT*) were measured. Fur Hg levels confirmed chronic MeHg intoxication. MeHg exposure raises TC levels both in wild-type and *APOE* ko mice. HDL and LDL-cholesterol levels were increased only in the MeHg-challenged *APOE* ko mice. MeHg increased liver lipid peroxidation, regardless of the genetic background. Unintoxicated *APOE* ko mice showed higher expression of *TERT* than all other groups. *APOE* deficiency increases *XPA* expression, regardless of MeHg intoxication. Furthermore, MeHg-intoxicated mice had more cytogenetic abnormalities, effect which was independent of *APOE* deficiency. More studies are needed to dissect the interactions between circulating lipids, MeHg intoxication, and DNA-repair pathways even at young age, interactions that likely play critical roles in cell senescence and the risk for chronic disorders later in life.

1. Introduction

Mercury (Hg) is one of the oldest chemical elements used in human health applications and of the most dangerous environmental pollutants (Berzas Nevado et al., 2010; Arrifano et al., 2018c). Human activities diversity and intensify the presence of this metal in the environment

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through processes such as burning fossil fuels, chlor-alkali industries, and traditional gold mining (Vriens et al., 2019; Wang et al., 2020; Crespo-Lopez et al., 2021). Despite knowledge about the dangers of mercury intoxication, populations are often ubiquitously exposed.

Methylmercury (MeHg) is a highly toxic organic form of Hg, produced mainly *via* methylating bacteria in aquatic sediments. In the food chain, humans can be intoxicated by contaminated fish intake, with predatory fish mainly being affected by MeHg biomagnification (Raposo et al., 2020). The highly toxic action of MeHg is explained by its ability to bind SH-tubulin, leading to inhibition of microtubule assembly, compromising the spindle function, and, finally, causing inhibition of cell growth (Ajsuvakova et al., 2020).

MeHg may lead to abnormalities in genetic material and DNA repair pathways (Cebulska-Wasilewska et al., 2005), as documented by *in vitro*, *in vivo*, and epidemiological studies (Crespo-López et al., 2009; Manzolli et al., 2015; Patnaik and Padhy, 2018). Some reports have shown that exposure to relatively low levels of MeHg has the potential to cause other long-term consequences, such as the induction of genotoxicity and tumorigenesis (Crespo-Lopez et al., 2016; Pieper et al., 2014).

Although Central Nervous System has been traditionally considered the main target of MeHg toxicity, deleterious cardiovascular consequences have also been highlighted, primarily due to chronic exposure (Leocádio et al., 2019; Hu et al., 2020).

Apolipoprotein E (ApoE = protein; *APOE* = gene) is critical for lipid metabolism and cholesterol reverse transport to the liver. The majority of ApoE is synthesized in the liver, and this organ is responsible for most of its production. ApoE also has various immune and inflammation modulatory roles and affects the progression of inflammatory-related chronic diseases (Arrifano et al., 2018c; Pereira et al., 2019). *APOE* knockout mice have been utilized to study the pathophysiology of atherosclerosis and neurodegenerative diseases in controlled laboratory conditions. The deficiency of ApoE leads to spontaneous dyslipidemia, atherosclerotic arterial lesions, and poor tissue repair, which are aggravated under high-fat diets (Liu et al., 2013).

Telomeres and telomerase reverse transcriptase (*TERT*) activity are cellular sentinels for environmental stressors based on their sensitivity to oxidative DNA damage and inflammation. Telomeres, hexameric repetitive DNA sequences at ends of chromosomes, are highly conserved from very primitive organisms to humans. Telomeres shorten during every cell cycle, but telomerase elongates the 3`-end of telomeres, contributing to genome stability (Zheng et al., 2019). In stem cells, telomerase expression is tightly regulated with deficient expression (Richardson et al., 2018). Telomere erosion in chromosomes is also a hallmark of cell tumorigenesis and cancer (Calado et al., 2009; Bajaj et al., 2020; Stone et al., 2016).

Chemical agents constantly damage the DNA, and the estimated numbers of single-strand breaks and spontaneous base losses in nuclear DNA are as high as 10^4/cells/day (Hoeijmakers, 2009). These DNA breaks are repaired by single-strand break repair (SSBR) or double-strand break repair mechanisms. For the single-strand break, one of the most effective mechanisms of repair is the nuclear excision repair (NER), which has two major pathways: global genome repair (GGR), probing the genome for strand distortions; and the transcription-coupled repair (TCR), which removes distorting lesions that block elongating RNA polymerases. The most important players of NER are the xeroderma pigmentosum complementation group, especially *XPA*, *XPC*, *XPD*, and *XPG*, being *XPC* the master of cell signaling for GGR (de Melo et al., 2016).

XRCC1 is another critical gene in which its protein interacts with multiple enzymatic components of SSBR, including DNA kinase, DNA phosphatase, DNA polymerase, DNA deadenylase, and DNA ligase. All these elements accelerate the repair of a broad range of DNA single-strand breaks. *XRCC1* has also been reported with a great relationship with poly(ADP-ribose)polymerase (PARP1) activity and has a critical role in preventing hereditary neurodegenerative disease (Horton et al., 2008; Caldecott, 2019).

This study aimed to evaluate the effects of chronic MeHg intoxication in young *APOE* knockout (ko) mice that spontaneously develop dyslipidemia, assessing systemic lipid parameters, liver oxidative stress, and the expression of DNA repair genes finally, the incidence of bone marrow chromosomal abnormalities. We hypothesize that MeHg intoxication and ApoE deficiency increase oxidative stress with reduced telomerase and xeroderma pigmentosum complementation group gene expression, thus predisposing to chromosomal abnormalities, which may augment the risk for cancer with aging.

2. Materials and methods

2.1. Animals

C57BL/6 J wild-type and *APOE* ko mice were obtained from the Experimental Biology Core vivarium at the University of Fortaleza. Mice were kept in microisolators with free access to water and food under strict temperature and humidity control. The experiments followed the Brazilian College of Animal Experimentation guidelines and were approved by the Animal Care and Use Committee from the University of Fortaleza (No. 4831110618).

A total of 60 male C57BL/6 J and *APOE* ko mice, aged 21 days, immediately after weaning, with an initial weight of 9–12 g, were included in the study. Experimental mice were divided into four age-matched experimental groups, as follows: 1) MeHg-challenged *APOE* ko mice; 2) unintoxicated-*APOE* ko controls; 3) MeHg-challenged wild-type mice; 4) unintoxicated wild-type controls.

All groups received water and food *ad libitum* for 40 days. At the end

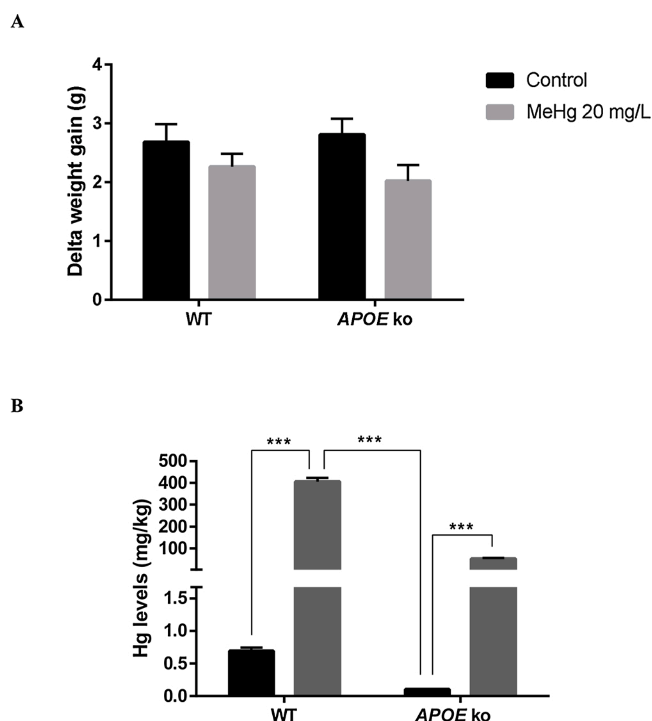

Fig. 1. A. Effect of MeHg intoxication on delta of body weight gain in C57BL/6 J wild-type (WT) (n = 8) and *APOE* ko mice (n = 8). There was no statistical difference between groups. Variations between rows p = 0.0799 and between columns p = 0.0975; Two-way ANOVA, Tukey post-test. **B.** Hair Hg concentration in C57BL/6 J wild-type (WT) (n = 6) and *APOE* ko mice following MeHg intoxication (20 mg/L in the drinking water for 20 days) (n = 6) and controls (n = 6). Values are expressed as mean ± standard error of the mean. ***p < 0.001. Variations between rows and between columns p < 0.0001; Two-way ANOVA, Tukey post-test.

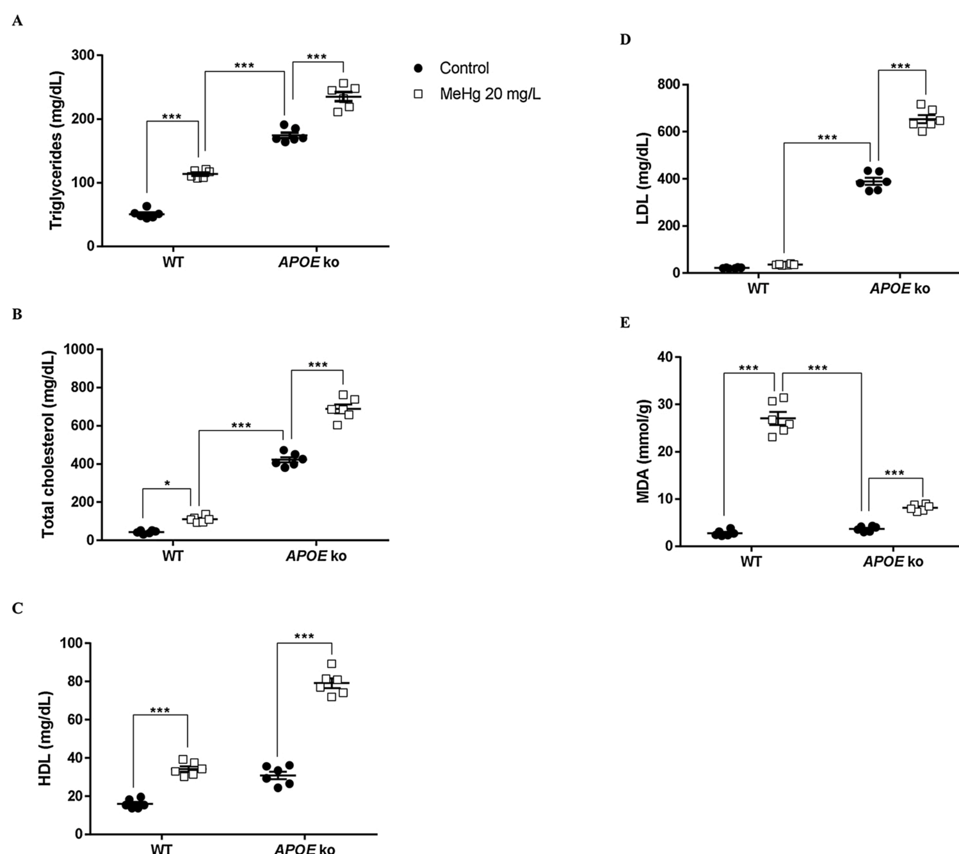

Fig. 2. Effect of MeHg intoxication on serum triglycerides (**A**), total cholesterol (**B**), HDL (**C**), LDL (**D**) levels, and liver malondialdehyde (MDA) levels (**E**) in C57BL/6 J wild-type (WT) (n = 6) and *APOE* ko mice (n = 6). ***p < 0.001. Values are expressed as mean ± standard error of the mean.

of the experimental protocol (61 days postnatal life), mice were euthanized with an overdose of ketamine/xylazine, and biological samples were immediately harvested.

2.2. MeHg chronic exposure

Challenged mice were exposed to MeHg daily, receiving a MeHg chloride (Sigma Aldrich, St. Louis, MI, USA) solution in the drinking water (20 mg/L) from postnatal day (PND) PND41 to PND61. This protocol is intended to mimic the oral ingestion of MeHg in humans. This MeHg dose has been found safe and causing chronic intoxication in previous studies (Lacerda Leocádio et al., 2020; Silva et al., 2020).

2.3. Body weight gain

In order to evaluate possible alterations in the bodyweight burden, we assessed the delta body weight gain, consisting of the difference between the final (PND61) and the initial body weight on day 41 at the onset of MeHg challenge (PND41).

2.4. Quantification of fur Hg levels

To address the level of MeHg chronic intoxication, we assessed mouse fur Hg levels and sought whether *APOE* ko mice would have more elevated levels. Fur samples were collected through trichotomy of the mouse lumbar region using plastic tweezers.

The determination of total Hg in the samples was performed using a Direct Mercury Analyser (DMA-80), Milestone by atomic absorption spectrometry. The technique involves the amalgamation of the released mercury, heating the amalgamation trap, and detecting elemental mercury (Windmöller et al., 2017). Results are shown in mg/Kg of the fur samples.

2.5. Analysis of plasma lipids levels (total cholesterol and triacylglycerols)

In order to analyze metabolic changes due to either compound or isolated effects of MeHg exposure and ApoE deficiency compared to controls, we harvested blood samples through cardiac puncture for determining total plasma cholesterol, triglycerides, LDL, and HDL-cholesterol levels using a semi-automatic analyzer and standard

diagnostic kits (Labtest®, Brazil).

2.6. Analyses of liver lipid peroxidation

Liver samples were obtained to determine tissue lipid peroxidation by assessing malondialdehyde (MDA) levels, as described elsewhere (Agar et al., 1999). Briefly, liver samples were weighed and homogenized in sodium phosphate buffer, in a proportion of 10 % (weight/-volume). Subsequently, the material was centrifuged at 10,000 rpm for 5 min at 4 °C, and the supernatant was collected, and 20 % acetic acid and 0.5 % thiobarbituric acid (TBA) were added. The solution was transferred to the water bath with a temperature of 95 °C for 1 h, under stirring every 15 min. After this period, the material was placed for 30 min in an ice bath, then an 8.1 % sodium dodecyl sulfate solution (SDS) was added and centrifuged at 12000 rpm, for 15 min, at room temperature. The supernatant was collected, and the sample was read on a spectrophotometer at 532 nm. The standard curve was obtained using 1,1,3,3-tetramethoxypropane as a standard. The results were expressed in nanomoles of malonaldehyde per milligram of tissue (nmol/mg of tissue).

2.7. Analyses of Xeroderma pigmentosum complementation group (XPA, XPC, XPD, and XPG), XRCC1, and TERT mRNA in the spleen

Spleen samples were immediately snap-frozen in liquid nitrogen and stored in a freezer −80 °C until analyses. Lysis and extraction of cellular RNA were performed using the mini-RNA extraction kit from Invitrogen™ (PureLink™ RNA Mini Kit). cDNA was obtained using the Applied Biosystems® Reverse Transcription Kit (High-capacity cDNA Reverse Transcription 50 Applied Biosystems® Kit). We evaluated the expression of *XPA*, *XPC*, *XPD*, *XPG*, *XRCC1*, and *TERT* mRNA for the four age-matched experimental groups. Quantitative real-time PCR (qPCR) reactions were based on TaqMan® methodology (Applied Biosystems, Carlsbad, CA, USA) and performed on a 7500 Fast System® (Applied Biosystems, Carlsbad, CA, USA). Pre-developed TaqMan gene expression assays for *XPA* (Mm0045711_m1), *XPC* (Mm01183434_m1), *ERCC2/XPD* (Mm00514776_m1), *ERCC5/XPG* (Mm01256322_m1), *XRCC1* (Mm00494222_m1), and *TERT* (Mm00436931_m1), as well as TaqMan Universal Master Mix II, with UNG® (Applied Biosystems, Carlsbad, CA, USA) were used to quantify mRNA expression *β-actin* (Mm00607939_s1) was used as the endogenous gene for normalization. Each sample was performed in duplicate, and the expression ratios were calculated using the $2^{-\Delta\Delta Cq}$ method. The Cq values were provided by the 7500 Fast Real-Time PCR System software (Applied Biosystems, Inc., Foster City, CA, USA).

2.8. Cytogenetic analysis

Conventional G-banded karyotype analyses were prepared from bone marrow cells (Guerra and de Souza, 2002) after euthanasia. Both femurs were immediately dissected, cut at both ends, and stored in a conical tube containing PBS and 1% penicillin/streptomycin (Gibco, Grand Island, NY, USA) until processing.

The marrow was flushed into a petri dish, and the material was aspirated and transferred to a conical tube with the complete culture medium containing RPMI 1640 medium (Gibco, Grand Island, NY, USA), 10 % of fetal bovine serum (FBS), 1% colchicine (final concentration 0.05 mg/mL) and 1 % Penicillin/Streptomycin (Gibco, Grand Island, NY, USA). The material remained in an incubator for 2 h at 37 °C with 5% carbon dioxide. After incubation, the cells were exposed to a KCl hypotonic solution (0.075 mol/L) and fixed with Carnoy's buffer (methanol/acetic acid in 3:1 proportion). The slides were prepared and stained with Wright staining. An average of 50 metaphases was analyzed for each group using CytoVision Automated Karyotyping System (Applied Imaging, San Jose, CA, USA).

2.9. Statistical analysis

Results were presented as mean ± standard error of the mean (SEM). Data on relative mRNA expression was presented with the fold-change by $2^{-\Delta\Delta}$Cq and confidence interval. Statistical difference between groups and conditions under investigation was tested by two-way analysis of variance (ANOVA) followed by the Tukey posthoc test using Graph pad Prism (GraphPad Prism 7 Software, San Diego, CA, USA). The difference was considered significant at P-value <0.05).

3. Results

3.1. Weight change and Mercury (Hg) fur levels

Neither MeHg chronic intoxication nor *APOE* deficiency alters delta weight gain in both wild-type and *APOE* ko mice ($p > 0.05$) (Fig. 1A) . MeHg-chronically exposed wild-type ($p < 0.0001$) and *APOE* ko ($p = 0.05$) mice showed a remarkable increase in fur Hg levels compared to unchallenged control groups (. Interestingly, *APOE* ko mice showed significantly lower fur Hg levels than wild-type counterparts ($p < 0.0001$) (Fig. 1B).

3.2. Systemic lipid parameters and liver oxidative stress

Chronic exposure to MeHg increased plasma triglycerides, total cholesterol, HDL, and LDL levels compared to controls, regardless of genetic background ($p < 0.0001$). In addition, *APOE* deficiency, regardless of MeHg intoxication, significantly raised all systemic lipid parameters ($p < 0.001$). Compounded *APOE* deficiency and MeHg intoxication marked increased lipid parameters compared to each alone (Fig. 2A-D) .

MeHg-intoxicated mice had higher MDA levels than controls, regardless of the genetic background (Fig. 2E) . Of note, the *APOE* ko mice showed less severe MeHg-induced liver lipid peroxidation than the challenged wild-type mice ($p < 0.0001$).

3.3. Xeroderma pigmentosum group

APOE ko mice showed higher splenic *XPA* expression compared to wild-type mice. Unchallenged *APOE* ko mice showed 2.6X higher mean expression of *XPA* than unchallenged wild-type mice ($p = 0.01$) and 3.3X higher mean expression than MeHg-intoxicated wild-type mice ($p = 0.006$). Challenged *APOE* ko mice showed 2.8X higher mean expression compared to MeHg-intoxicated wild-type mice ($p = 0.04$).

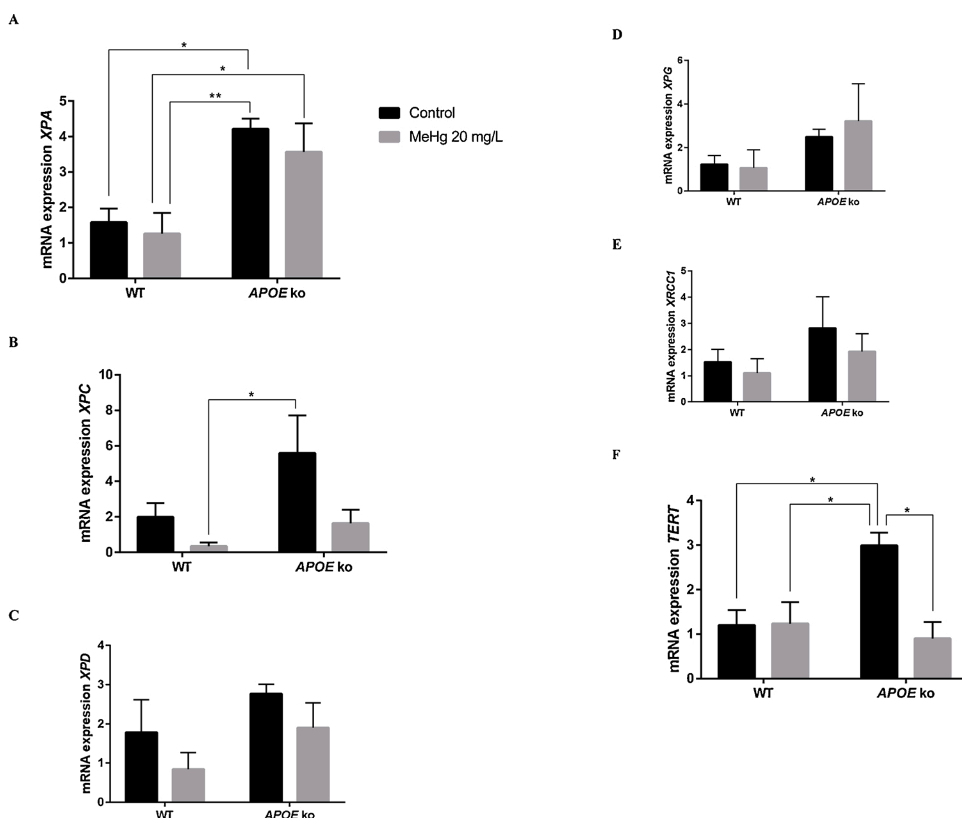

Fig. 3. MeHg effects on transcriptional levels of xeroderma pigmentosum, complementation group A (*XPA*) (**A**) (n = 7) *p < 0.05; **p < 0.01. Variations between rows p = 0.0002; Two-way ANOVA, Tukey post-test. *XPC* (**B**) (n = 7) *p < 0.05. Variations between rows and between columns p < 0.05; Two-way ANOVA, Tukey post-test. *XPD* (**C**) (n = 5); there were no statistically significant differences. Two-way ANOVA, Tukey post-test. *XPG* (**D**) (n = 4); there were no statistically significant differences. Two-way ANOVA, Tukey post-test. X-ray repair cross-complementing protein 1 (*XRCC1*) (**E**) (n = 7); There were no statistically significant differences. Two-way ANOVA, Tukey post-test. And telomerase reverse transcriptase (*TERT*) in the spleen (**F**) (n = 5); *p < 0.05. Variations between Rows p = 0.0792 and between columns p = 0.0185; Two-way ANOVA, Tukey post-test. Values are expressed as mean ± standard error of the mean.

Unchallenged *APOE* ko mice showed a marked 16X higher *XPC* expression compared to challenged wild-type mice (p = 0.015). No statistical significance was found regarding *XPD* and *XPG* analyses (p > 0.05) (Fig. 3A-D) .

3.4. X-ray cross complimentary repair protein (XRCC1)

Intoxicated animals showed less expression of *XRCC1*, but without reaching statistical significance (p > 0.05) (Fig. 3E) .

3.5. Telomerase reverse transcriptase (TERT)

Statistically significant differences were observed in *TERT* mean expression in *APOE* ko non-intoxicated mice compared to the other groups. Unchallenged *APOE* ko animals showed 2.5X higher mean expression of *TERT* compared to wild-type non-intoxicated mice (p = 0.029), 2.4X higher mean expression compared to MeHg-intoxicated wild-type mice (p = 0.03) and 3.3X higher mean expression compared to challenged *APOE* ko mice (p = 0.01) (Fig. 3F) .

3.6. Cytogenetic analysis

Successful cytogenetic analyses were performed in 19 mice, and an average of 50 metaphases was analyzed for each group. We detected 33 chromosomal translocations. The non-intoxicated wild-type group presented six translocations, while the MeHg-intoxicated wild-type group showed 12 translocations. The non-intoxicated *APOE* ko group presented five translocations, while the MeHg intoxicated *APOE* ko group

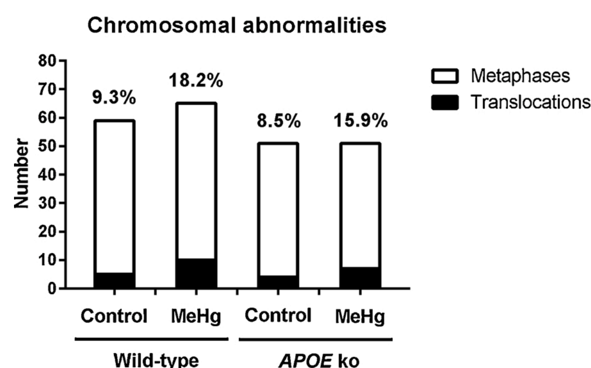

Fig. 4. Representative panel of mostly found bone marrow chromosomal translocations following MeHg intoxication (20 mg/L in the drinking water for 20 days) from C57BL/6 J wild-type (WT) (n = 10) and *APOE* ko mice (n = 9).

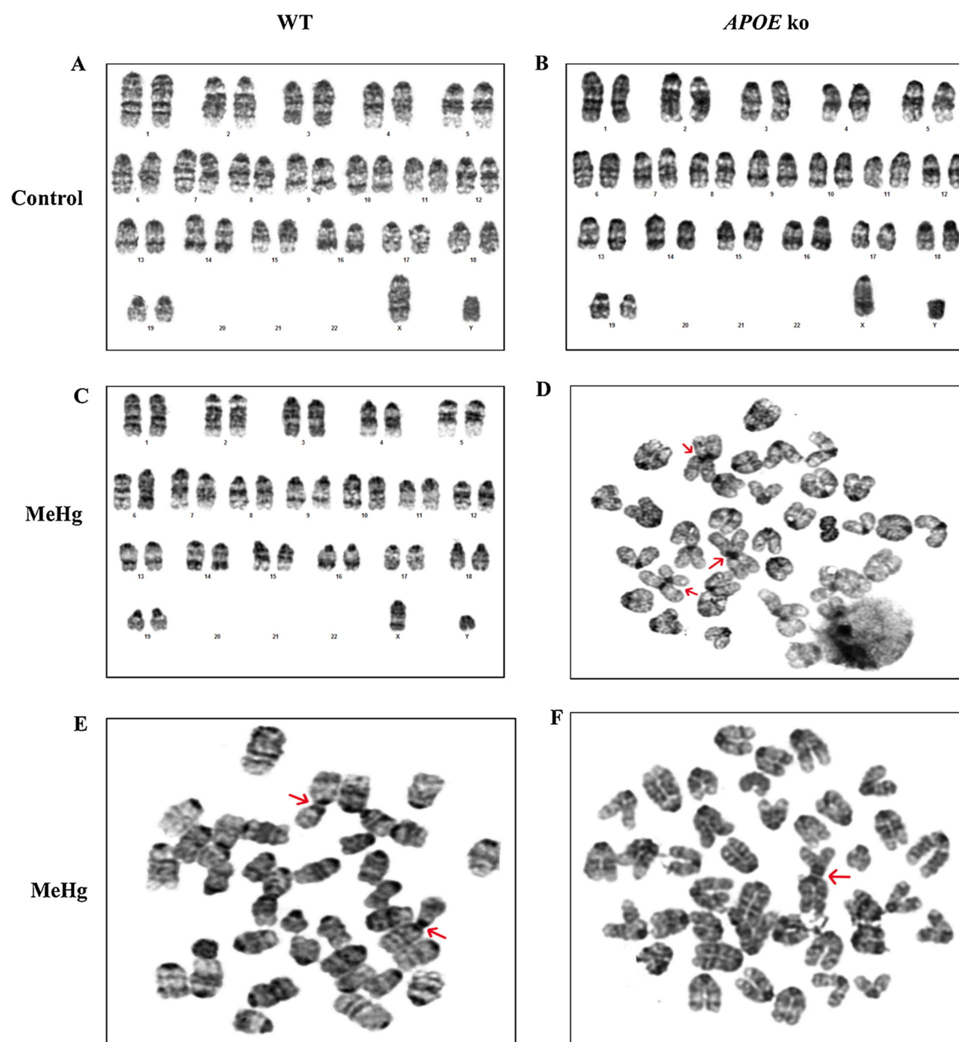

Fig. 5. Representative panel of bone marrow karyotypes obtained from chromosomal metaphases of wild-type (WT) mice, n = 5, and *APOE* ko controls, n = 4, (**A and B**) and of wild-type (WT) mice, n = 5, and MeHg-intoxicated *APOE* ko mice, n = 5 (**C-F**). Chromosomal translocations are indicated by red arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

showed ten translocations. These results suggest that MeHg is responsible for translocations among intoxicated groups independent of the genetic background (Figs. 4 and 5).

4. Discussion

As far as we know, this is the first study to evaluate MeHg genotoxic effects in *APOE* ko mice that are spontaneously dyslipidemic even at a young age. We assessed the expression of DNA repair genes *(XPA*, *XPC*, *XPD*, *XPG*, and *XRCC1)* and *TERT* (telomerase reverse transcriptase), the latter a key factor regulating cell replicative senescence and tumor progression (Trybek et al., 2020). Altered cell senescence may be related to increased oxidative stress (Kamal et al., 2020). Impaired oxidative balance has been recognized with MeHg intoxication in mice (Fujimura and Usuki, 2017). In addition, cell replicative senescence has been found in neural stem cells following MeHg exposure (Bose et al., 2012).

In our study, wild-type mice challenged with MeHg in drinking water showed remarkably higher fur Hg levels than the *APOE* ko counterparts, suggesting that the lack of ApoE affects mercurial intestinal absorption and bioavailability. MeHg may accumulate in body fat from ApoE ko mice, which are known to have increased total visceral and mesenteric fat (Yamamoto et al., 2014; Park et al., 2017). In addition, altered intestinal microbiota may facilitate MeHg demethylation and fecal excretion. Intestinal bacterial populations have been found to demethylate MeHg (Pinto et al., 2020; Lin et al., 2020). Another more plausible possibility (as MeHg intoxication could not induce obesity in our model) is that MeHg binds to circulating lipoproteins and presumably more in HDL fractions. Early studies from Reichert and Malins suggest that Hg could bind to HDL in salmon fish (Reichert and Malins, 1974, 1975), an effect which presumably could likewise happen in mice. Marked MeHg intoxication could drive liver altered function and increased HDL release to prevent systemic tissue injury. This hypothesis would need further studies to be confirmed.

Our current data further support that MeHg intoxication per se could induce dyslipidemia, as previously found by our group in young wild-type mice (Lacerda Leocádio et al., 2020), which is even worse in baseline line dyslipidemic *APOE* ko mice. This finding is important, as long-term effects of MeHg exposure may be more harmful to individuals with hypercholesterolemia, especially in emerging countries coping with the nutritional transition with the dual burden of malnutrition and over-nutrition in Brazil.

In our study, MeHg-challenged mice, regardless of genetic background, showed high total cholesterol plasma levels than unintoxicated controls, corroborating previous results (Silva et al., 2020). *APOE* ko mice, which are naturally dyslipidemic animals, showed marked LDL cholesterol levels, roughly ten times higher than HDL cholesterol levels when exposed to MeHg.

Studies in riverside populations in the Amazon, historically exposed

to MeHg due to contaminated fish, have found altered systemic blood pressure, hypercholesterolemia (Arrifano et al., 2018c; Arrifano et al., 2021), also related to *APOE*4 genotypes (Arrifano et al., 2018b). *APOE*4 is a well-known genetic risk factor for neurodegeneration and non-communicable diseases like hypertension and diabetes (Di Battista et al., 2016; Arrifano et al., 2018c).

A role for apolipoprotein E was proposed in mercury toxicity, suggesting a toxicodynamic influence (Arrifano et al., 2018a). Still, recent epidemiological data pointed to the possible existence of a toxicokinetic influence with the *APOE*4 genotype accumulating more mercury (Arrifano et al., 2018b). Moreover, the most recent data reveal that oral methylmercury intoxication aggravates cardiovascular risk factors and accelerates atherosclerosis lesion development in ApoE ko animals (Silva et al., 2020).

Recent data point to an additional alteration caused by oral exposure to MeHg with possible long-term consequences. Similar to exposed populations, mercury exposure was associated with decreasing RNA expression of telomerase reverse transcriptase (*TERT*), revealing the metal's impact in this marker of longevity (Crespo-López et al., 2019).

Our data show that MeHg-challenged mice exhibited increased oxidative stress. Wistar rats treated with 5 mg/kg/day MeHg and 1 mg/kg/day of diphenyl diselenide, intragastrically for 21 days, showed increased accumulation of Hg in the liver and the brain, leading to motor deficits and body weight loss (Dalla Corte et al., 2013). Increased MeHg accumulation in the liver may cause liver dysfunction and altered circulating plasma lipids seen in our study.

Other known toxic effects of MeHg are caused by the increased production of reactive oxygen species and increased oxidative stress, a mechanism that has been well-studied in different experimental conditions and tissues (Eom et al., 2014). Our findings corroborate several studies that show the increase of oxidative stress to MeHg. As detected by MDA levels, chronic MeHg exposure increased lipid peroxidation in the liver, regardless of the genetic background. Of note, intoxicated *APOE* ko mice had significantly lower MDA hepatic levels than the MeHg-challenged wild-type mice, which may be explained by reduced MeHg bioavailability to the liver with ApoE deficiency. Conversely, ApoE-cholesterol complex in triglyceride-rich lipoproteins may facilitate MeHg transport to the liver. This speculative mechanism requires further investigation.

APOE ko mice are dyslipidemic even at a young age. Dyslipidemia is considered a significant etiologic factor for cancer development later in life (Braun et al., 2011). According to IARC, dyslipidemia and obesity have been consistently associated with an increased chance of developing multiple myeloma, lymphoma, and other tumors in humans (Lauby-secretan et al., 2016). As dyslipidemia and obesity increase oxidative stress, DNA damage may emerge with single or double-strand breaks, predisposing to chromosomal abnormalities, the hallmark of tumorigenesis. The increased expression of selected DNA repair genes in *APOE* ko mice seen in our study may be a compensatory response to presumably augmented DNA damage caused by the dyslipidemic profile, even without obesity (Folkmann et al., 2007).

Unchallenged *APOE* ko mice have higher splenic gene expression of *TERT* than all the other groups. Increased *TERT* may compensate for cell senescence due to *APOE* deficiency, independent of MeHg intoxication. Dyslipidemia seems to be a strong driving factor to DNA gene repair activation. Such lipid interactions remain unclear and require further investigation.

Dyslipidemia may increase oxidative stress on fat cells and shortens telomeres, which may be a consequence of a reduced action of *TERT* (Ahima, 2009). The close relationship between *TERT* RNA expression and oxidative stress in the context of MeHg intoxication was recently documented, where the potent antioxidant fruit *Euterpe oleracea* was able to prevent oxidative stress and significantly decrease *TERT* RNA expression with no effects on mercury accumulation (Crespo-Lopez et al., 2019). Furthermore, TERT-deficient mice showed less neointimal formation in a guidewire-induced arterial injury model (Endorf et al., 2017).

The higher number of chromosomal abnormalities among the intoxicated animals of each group, wild-type and *APOE* ko, concerning the respective control groups demonstrate MeHg induces genomic instability. Translocation can occur when segments between two chromosomes are exchanged, then called reciprocal translocation. There are also non-reciprocal translocations (also known as Robertsonian) occurring when two acrocentric fuses near the centromeric region reduce chromosomal numbers (Nambiar et al., 2008). Previous studies using either methylmercury or mercuric chloride (HgCl2) have shown several chromosomal abnormalities, including deletions, duplications, and other numerical changes and chromatin disorders (Silva-Pereira et al., 2005). In humans, the aberrant expression of *TERT* has been linked to chromosomal abnormalities and cancer (Hartmann et al., 2005; Nagel et al., 2010).

5. Conclusions

Altogether our findings suggest that the compound effect of ApoE deficiency and dyslipidemia in *APOE* ko mice, even at a young age, causes greater expression of *XPA,* a nucleotide excision DNA repair-related gene. In addition, our results indicate that MeHg alone decreased the gene expression level of *XPC*. Of note, only *APOE* deficiency markedly increases *TERT* gene expression.

One limitation of our study is that the gene expression data need to be coupled with tissue protein levels to dissect the interactions with MeHg further, circulating lipid levels, and other players involved in DNA repair pathways. These studies are underway in our laboratory.

Our data suggest that *APOE* ko animals show less toxic effects compared to wild-type counterparts upon MeHg intoxication and MeHg toxicity was not related to *APOE* expression. Importantly, MeHg intoxication contributes to marked chromosomal alterations, reinforcing its genotoxic effects in wild-type and ApoE deficient young mice. Such effects raise more awareness of the risk of cancerogenesis in chronically MeHg-exposed human populations with aging.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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