REDOX EXPERIMENTAL MEDICINE

Redox Experimental Medicine (2024) **2024** e240010
https://doi.org/10.1530/REM-24-0010

Received 13 August 2024
Accepted 1 October 2024
Available online 1 October 2024
Version of Record published 25 October 2024

RESEARCH

Evidence of altered redox homeostasis in trichothiodystrophy

Valeria Cordone[1,*], Anna Guiotto[1,2,*], Vivian Bucay[3], Alessandra Pecorelli[1,4] and Giuseppe Valacchi[1,2,5]

[1]Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy
[2]Department of Animal Science, Plants for Human Health Institute, North Carolina State University, Kannapolis, North Carolina, USA
[3]Bucay Center for Dermatology and Aesthetics, San Antonio, Texas, USA
[4]Department of Food, Bioprocessing and Nutrition Sciences, Plants for Human Health Institute, North Carolina State University, Kannapolis, North Carolina, USA
[5]Department of Food and Nutrition, Kyung Hee University, Seoul, South Korea

Correspondence should be addressed to G Valacchi: gvalacc@ncsu.edu

*(V Cordone and A Guiotto contributed equally to this work)

Abstract

Objective: Trichothiodystrophy (TTD) is a rare hereditary disease whose prominent feature is brittle hair. Additional clinical signs are physical and neurodevelopmental abnormalities and in about half of the cases hypersensitivity to UV radiation. Although the mutations involved in this condition have been characterized, the correlation between the molecular defects and the plethora of clinical symptoms is not well understood. Recently, the presence of a redox imbalance in TTD has been suggested although no clear evidence has been reported on this aspect.

Methods: In the present study, we evaluated the redox status of fibroblasts isolated from a TTD patient. In addition, to understand the ability of TTD cells to respond to oxidative insults, the cells were challenged with H_2O_2. Mitochondrial $O_2^{\bullet-}$ and mitochondrial membrane potential were measured in different oxidative conditions. In addition, protein levels of NRF2 and BACH1 were also analyzed in response to H_2O_2.

Results: The results suggested an aberrant mitochondrial response to oxidative stimuli, an increased baseline oxidative stress status in TTD, and an altered NRF2/BACH1 level.

Conclusions: This study emphasizes the altered redox homeostasis in TTD pathogenesis and mitochondria functionality.

Significance statement

Focusing on mitochondria homeostasis and redox imbalance could represent an alternative therapeutic target for this condition to improve patients' clinical features.

Keywords: BACH; mitochondria; NRF2; trichothiodystrophy

Published by Bioscientifica Ltd.
https://rem.bioscientifica.com
© 2024 the author(s)

This work is licensed under a Creative Commons Attribution 4.0 International License.

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/

Redox Experimental Medicine (2024) **2024** e240010
https://doi.org/10.1530/REM-24-0010

Introduction

Trichothiodystrophy (TTD) is a rare autosomal recessive multisystem disorder, characterized by brittle nails and hair due to keratinocytes lacking sulfur-rich proteins (Itin *et al.* 2001). Particularly, by observing the hair from TTD subjects under polarising microscopy, it shows an alternating light and darkness banding pattern, termed 'tiger tail banding', due to the reduced cysteine amount in the hair (in TTD is less than 50% of the normal content) (Liang *et al.* 2005). Indeed, the name of the pathology recalls this feature (from Greek '*tricho*', hair; '*thio*', sulphur; '*dys*', faulty; '*trophe*', nourishment). The incidence rate for TTD is approximately 1 per million live births, and the prevalence rate has been estimated at 1 per 830,000 in Europe (Kleijer *et al.* 2008).

A wide range of other clinical characteristics with variable degrees of severity can be seen in TTD individuals, i.e. physical and mental retardation, microcephaly, small stature, unusual facial features, ichthyotic skin, decreased fertility, cataracts, recurrent infections, anemia, and signs of premature aging (Faghri *et al.* 2008). Skin photosensitivity is also described in about 50% of TTD patients (PS-TTD) and is associated with defects in nucleotide excision repair (NER) of UV-damaged DNA (Orioli & Stefanini 2019). Although the NER deficiency (the most common mutations involve *ERCC2* – or *XPD* – gene) in TTD is indistinguishable from that reported in Xeroderma Pigmentosum type D, TTD patients do not show augmented risks of skin cancers (Wijnhoven *et al.* 2005, Orioli & Stefanini 2019).

Non-photosensitive TTD (NPS-TTD) is characterized by biallelic mutations in genes not implied in DNA repair processes, like MPLKIP, RNF113A, GTF2E2, CARS1, TARS1, MARS1, and AARS1 (Nakabayashi *et al.* 2005, Corbett *et al.* 2015, Kuschal *et al.* 2016, Kuo *et al.* 2019, Theil *et al.* 2019, Botta *et al.* 2021). For instance, a recent paper highlighted the role of RNF113A in modulating the redox cellular balance, bringing up the idea that possible oxidative stress (OS) might play a role in this condition (Cho *et al.* 2024).

In fact, despite the known genetic mutations of both PS and NPS-TTD, the correlation between the molecular defects and the plethora of clinical symptoms is poorly understood and the idea that a corrupted redox homeostasis is involved in this pathology has been hypothesized.

The term 'oxidative stress' describes a series of events, leading to damage to biological macro-molecules, as a consequence of an imbalance between cellular antioxidant defence and reactive oxygen species (ROS) production. OS is a key player in aging (Maldonado *et al.* 2023), as well as a large number of human pathologies, including neurological diseases (Pecorelli *et al.* 2011, Dias *et al.* 2013, Liu *et al.* 2022), cancer (Hayes *et al.* 2020, Arfin *et al.* 2021), inflammatory bowel disease (Li *et al.* 2023).

As known, ROS can be produced during cellular metabolism, inflammatory responses or exposure to physical and chemical agents (Markkanen 2017). Recently, Lerner and colleagues showed that *XPD*-mutated primary fibroblasts from TTD subjects presented high basal levels of intracellular ROS (Lerner *et al.* 2019). In addition, it has been demonstrated that NER may be implicated in the repair of lesions generated by ROS, like 8-oxo-7,8-dihydroguanine (8-oxoG) (Shafirovich *et al.* 2016).

In light of this, the present work was designed to characterize and evaluate the redox homeostasis, mitochondrial functionality, and the triggering of an appropriate antioxidant response in an *ex-vivo* TTD model. For these purposes, skin fibroblasts deriving from one TTD patient ($n = 1$) were used as they represent a good model to study the molecular mechanisms involved in neurological disorders, as well as multi-system broad-spectrum pathologies (Auburger *et al.* 2012, Cervellati *et al.* 2015, Cordone *et al.* 2019).

Materials and methods

Human skin fibroblast isolation and culture

Primary fibroblasts were isolated from a 3-mm skin biopsy from one healthy and one TTD subject, as previously described (Sticozzi *et al.* 2013, Cordone *et al.* 2019). Cells were kept in culture with low glucose-DMEM medium (cat. 11054020, Thermo Fisher Scientific Inc.) supplemented with fetal bovine serum (10% v/v), L-glutamine (2 mM) and antibiotics (100 IU/mL penicillin, 100 µg/mL streptomycin) (cat. 26140079, 25030081 and 15140122, Thermo Fisher Scientific Inc., respectively) and incubated at 37°C in a humidified atmosphere (5% CO_2). All the subsequent experiments were performed by using fibroblasts between the sixth and tenth passage *in vitro*.

Human skin biopsies were obtained from biological waste material during diagnostic procedures of skin cancer and represented normal tissues adjacent to cancer tissues. Tissue collection was carried out in compliance with the Declaration of Helsinki of the World Medical Association and signed written informed consent.

Cell treatments

For the experiments reported in this study, control and TTD cells were starved for 12 h with 1% FBS-supplemented medium. Subsequently, fibroblasts were treated for 1, 6, and 24 h with 100 µM of hydrogen peroxide (H_2O_2) (cat. H1009, Sigma-Aldrich), as previously reported (Pecorelli *et al.* 2015, 2020*a*). In the experiments with MitoTEMPO (cat. SML0737, Sigma-Aldrich), cells were first pre-treated with 1 µM of MitoTEMPO for 2 h, and then the surnatant was

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/

Redox Experimental Medicine (2024) **2024** e240010
https://doi.org/10.1530/REM-24-0010

replaced with H_2O_2- and MitoTEMPO-containing medium for the indicated time-points, as described above.

Oxidants production analysis

The analysis of oxidant production was performed by reactive oxygen species (ROS) Detection Reagents (cat. MP36103, Invitrogen), as previously described (Pasqui *et al.* 2024). After the overnight starvation, fibroblasts were incubated in darkness with 2',7'-dichlorofluorescein diacetate (DCFDA) in 1% FBS-containing medium for 30 min at 37°C in a humidified 5% CO_2 atmosphere. At the end of the incubation, the medium was removed, and fibroblasts were washed once with D-PBS in order to eliminate the excess of the probe. Then, the cells were incubated for 1, 6, and 24h with new media containing the treatments. At the end of each treatment, DCF fluorescence, as a measure of oxidant production, was determined by SpectraMax iD3 plate reader (Molecular Devices, LLC., San Jose, CA, USA), at 485 nm (excitation filter) and 530 nm (emission filter). Data were normalized against cell density determination, performed by sulforhodamine B (SRB) staining.

Mitochondrial ROS measurement

Mitochondrial ROS production was evaluated by confocal microscopy using the specific probe MitoSOX™ Red (cat. M36008, Thermo Fisher Scientific Inc.). According to the manufacturer's instructions, fibroblasts were seeded on coverslips, and after treatments, they were incubated for 10 min at 37°C in HBSS buffer containing a final concentration of 5 µM of MitoSOX™ Red probe. Cells were then washed with D-PBS three times and fixed for 10 min with 10% neutral buffered formalin at room temperature. The washes with D-PBS were repeated two times, and the nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (cat. D1306, Thermo Fisher Scientific Inc.) (1:50,000 dilution in D-PBS) for 3 min. The coverslips were then mounted on microscope slides using the Fluoromount-G mounting medium (cat. 5018788, Thermo Fisher Scientific Inc.). Images were acquired by Zeiss LSM 710 confocal microscope (Carl Zeiss) with a 40× objective and analyzed using Java-based Fiji-ImageJ software and reported as mean fluorescence intensity (Cordone *et al.* 2022).

Mitochondrial membrane potential assay

An evaluation of mitochondrial membrane potential (MMP, ΔΨm) was performed by the specific probe MitoTracker® Orange CMTMRos (cat. M7510, Thermo Fisher Scientific Inc.). Cells were seeded and grown on coverslips inside a 24-well plate filled with the appropriate culture medium. At the end of the treatments, a new medium containing a final concentration of 100 nM of the probe was added to the cells, and incubated for 45 min at 37°C (5% CO_2). The cells were then quickly washed with PBS and fixed for 10 min at room temperature with 10% neutral buffered formalin. After fixation, the cells were washed three times with D-PBS, the nuclei were stained with DAPI, and the coverslips were mounted onto glass, as reported above. Images at 40× magnification were collected immediately by Zeiss LSM 710 confocal microscope (Carl Zeiss). Results were analyzed by Java-based Fiji-ImageJ software and given as mean fluorescence intensity (Kholmukhamedov *et al.* 2013, Neikirk *et al.* 2023).

C11-BODIPY™ Lipid Peroxidation (LPO) Assay

The detection of lipid peroxidation in TTD and CTRL live fibroblasts was performed by using BODIPY™ 581/591 C11, a sensitive fluorescent probe able to localize into live cell membranes (cat. D3861, Thermo Fisher Scientific Inc.) (Vadarevu *et al.* 2021). Briefly, 40,000 cells were plated on 12-mm coverslips placed in a 6-well plate and incubated overnight in 1% FBS-supplemented medium, then treated with hydrogen peroxide, as described in the 'Cell treatments sub-section. At the end of the treatments, the medium was replaced with a new medium containing a final concentration of 1 µM of BODIPY™ 581/591 C11 and incubated for 30 min in the incubator. Subsequently, three washes with D-PBS were performed and the fibroblasts were fixed with 10% neutral buffered formalin at room temperature for 10 min. Fibroblasts were then washed with D-PBS, and incubated with DAPI solution, as previously described. Finally, the cells were mounted onto microscope slides. 40×-magnification images were immediately acquired by Zeiss LSM 710 confocal microscope (Carl Zeiss). The levels of lipid peroxidation were determined by monitoring the increase of the green fluorescence intensity (emission at 510 nm). Digital images were analyzed by using Java-based Fiji-ImageJ software. Results were shown as mean fluorescence intensity.

Immunofluorescence analysis

Cells were seeded in a 6-well plate containing coverslips. After the overnight starvation and the H_2O_2 treatments, cells were washed twice with D-PBS, then fixed in 10% neutral buffered formalin for 10 min at room temperature, and permeabilized with D-PBS containing 0.25% (v/v) Triton X-100 for 10 min at 4°C, as previously described (Pecorelli *et al.* 2020*b*). Non-specific binding sites were blocked with 1% (w/v) bovine serum albumin (BSA, cat. A7906, Sigma-Aldrich) in D-PBS for 45 min. Cells were then incubated overnight with primary antibodies diluted in 0.25% (w/v) BSA-containing D-PBS at 4°C. The following primary antibodies were used: anti-BACH1 (cat. HPA034949, Sigma-Aldrich, dilution 1:250); anti-NRF2 (cat. sc-365949, Santa Cruz Biotechnology, dilution 1:100); anti-4-hydroxynonenal (4-HNE) (cat. AB5605, Merk Millipore, dilution 1:400); and anti-p-H2AX (cat. sc-517348, Santa Cruz Biotechnology, dilution 1:100). The

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution
4.0 International License.
http://creativecommons.org/licenses/by/4.0/

Redox Experimental Medicine (2024) **2024** e240010
https://doi.org/10.1530/REM-24-0010

day after, cells were washed with D-PBS, and incubated with solutions of fluorescent dye-cross adsorbed secondary antibodies (cat. A-11008, Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488; cat. A-11004, Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568; cat. A-11057, Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568, Thermo Fisher Scientific Inc., dilution 1:1,000) in 0.25% BSA-containing D-PBS for 1 h at room temperature. After further washes with D-PBS and the counterstaining with DAPI, the coverslips were mounted onto microscope slides and photographed by Zeiss LSM 710 confocal microscope (Carl Zeiss) at 40× magnification. The analysis was performed using Java-based Fiji-ImageJ software. Results were given as mean fluorescence intensity.

Statistical analysis

Statistical analyses were performed by using Statsoft Statistica10 and GraphPad Prism 8 software. Data were expressed as means ± standard error of the mean (S.E.M.) and are the results of three different cultures from cells of n = 1 control subject and n = 1 TTD patient. Factorial analysis of variance (ANOVA), with *post-hoc* Tukey's tests was applied. A P value of <0.05 was regarded as statistically significant for all the tests.

Results

TTD fibroblasts oxidative response to H_2O_2 challenges

In order to investigate the possible impairment of redox homeostasis in TTD primary fibroblasts, we first stimulated cells with H_2O_2 (100 µM) and then evaluated both oxidants production (Fig. 1A) and superoxide anion levels (Fig. 1B and C).

In particular, it was observed that TTD cells presented higher levels of oxidants already in basal condition, as compared to basal CTRL fibroblasts (Fig. 1A). As expected, the H_2O_2-challenge was able to trigger an increased oxidants generation in CTRL cells, starting after 1h of exposure (as compared to baseline CTRL). In TTD fibroblasts the treatment with H_2O_2 was able to further significantly augment the signal related to oxidants, with respect to the CTRL cells at all the considered time-points (1h, 6h, and 24h of H_2O_2 treatment) (Fig. 1A).

We then analyzed the mechanistic role of mitochondrial ROS (mtROS) in primary TTD fibroblast response, challenged with H_2O_2, by using MitoTEMPO, a mitochondrial-targeted antioxidant and specific scavenger of mitochondrial superoxide (Peoples *et al.* 2019). The generation of mtROS, mainly superoxide anion, measured by MitoSOX-related fluorescence levels (Fig. 1B) was enhanced in basal TTD cells, as compared to CTRL, and raised after 1h-, 6h- and 24h-exposure to H_2O_2 in both CTRL and TTD cells, with a higher fluorescence signal in treated TTD cells (Fig. 1C). The treatment with MitoTEMPO for 6 and 24h in TTD cells led to a decrease of the mtROS production, to the levels of CTRL cells. In addition, a significant reduction of mtROS upon H_2O_2-treatment was achieved by 1h-MitoTEMPO exposure in CTRL cells (^{+++}P < 0.01 vs CTRL+H_2O_2 1 h), while only after 6h-MitoTEMPO stimulation in TTD fibroblasts (Fig. 1B and C).

Taken together, these results showed an unbalanced redox homeostasis in TTD primary cells, with a greater response in terms of oxidants generation to the pro-oxidant challenge, as compared to control fibroblasts. Furthermore, the mitochondria seemed to play a key role in this redox impairment, since MitoTEMPO differently affected H_2O_2-induced mtROS generation in CTRL and TTD cells.

Reduced mitochondrial membrane potential in fibroblasts isolated from a TTD patient

In homeostatic conditions, between the inner and outer mitochondrial membranes, an electrochemical gradient ensures the ATP production and the maintenance of the mitochondrial membrane potential (MMP, ΔΨm), which is in turn essential for the correct mitochondrial activities (e.g. preservation of the electrochemical potential of hydrogen ions, as well as the process of energy storage during oxidative phosphorylation) (Zorova *et al.* 2018).

Since an over-generation of mtROS may often be the result of mitochondrial impairments/dysfunctions (Guo *et al.* 2013, Palma *et al.* 2024), we wanted to evaluate MMP in CTRL and TTD cell models, as an indicator of mitochondrial integrity/homeostasis.

In particular, we found that H_2O_2 was able to induce in CTRL cells a strong decrease in the MMP after 1 and 6 h of exposure, while upon 24h-treatment this response seemed to return to the basal condition (Fig. 2A and B). In TTD fibroblasts, ΔΨm was constitutively lower than non-treated CTRL cells (P < 0.001), with levels of MMP comparable to those observed in CTRL cells challenged with H_2O_2 (for 1 and 6 h) (Fig. 2B). In addition, we did not notice any substantial response in terms of ΔΨm in TTD cells, following H_2O_2 exposure at all the considered time-points (vs basal TTD). Indeed, also after 6 and 24h-H_2O_2 challenge TTD cells presented a reduced MMP, as compared to CTRL cells stimulated for the same time (Fig. 2B).

Altogether, our data seemed to indicate an alteration in mitochondrial function of TTD cells, both in basal and after a pro-oxidant stimuli. This impaired mitochondrial response was consistent with the augmented mtROS generation in TTD fibroblasts (as reported in Fig. 1B and C), suggesting a possible vicious circle in mitochondrial dysfunction.

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/

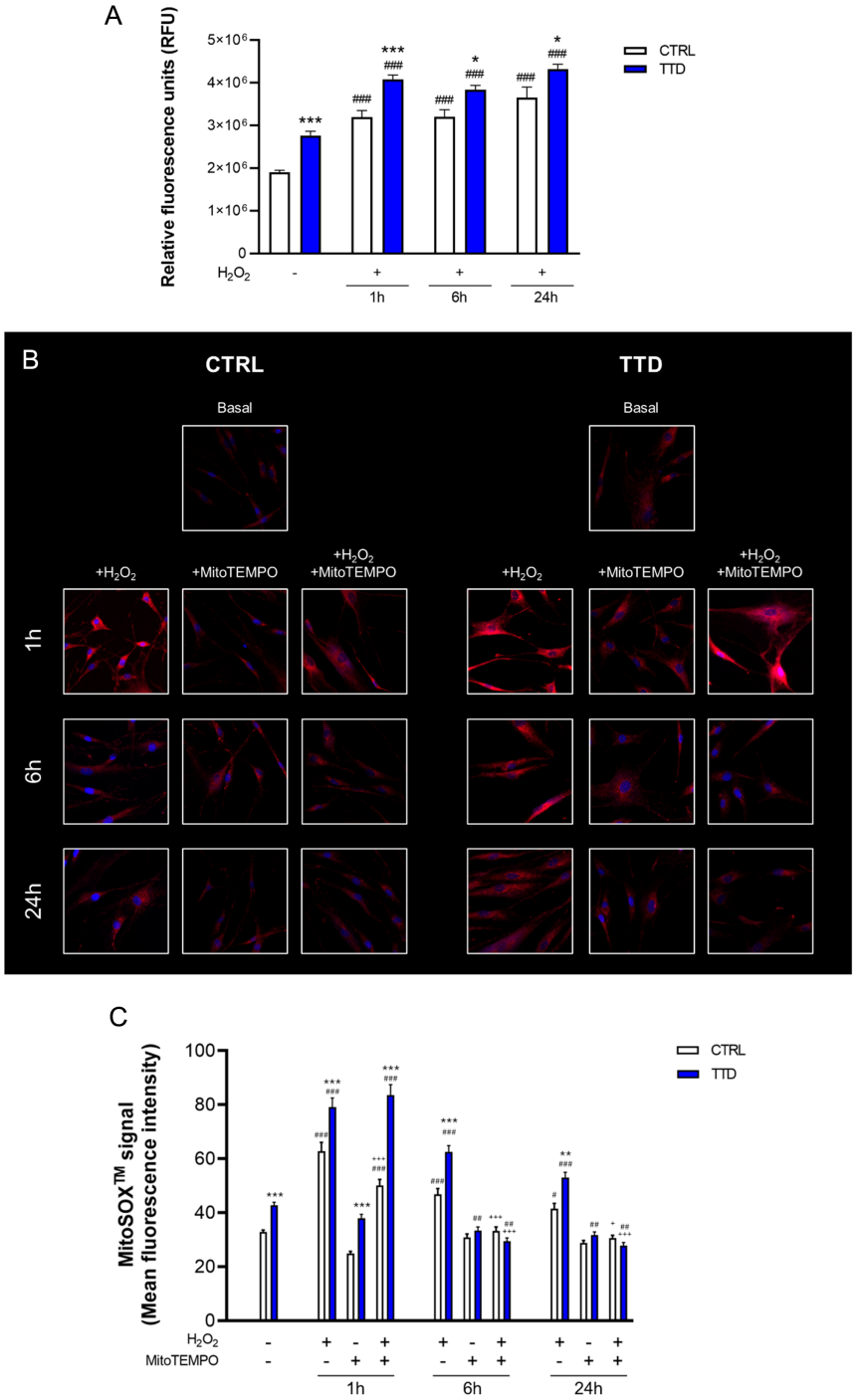

Figure 1

Overall oxidant production and mitochondrial ROS levels in TTD fibroblasts. (A) Levels of total oxidants in control and TTD fibroblasts in basal condition or exposed to hydrogen peroxide challenge (100 μM) for 1h, 6h and 24h, assessed by DCFDA assay. (B) Representative images and (C) analysis of MitoSOX™ Red fluorescence staining in control and TTD fibroblasts, pre-treated with MitoTEMPO (1 μM for 2 h) and then co-incubated or not with hydrogen peroxide (100 μM) for 1 h, 6 h, and 24 h. Nuclei are counterstained with DAPI. Objective 40×. Data were expressed as means ± S.E.M., and are the result of three different cultures of cells from $n = 1$ control subject and $n = 1$ TTD patient. *indicates significant differences between CTRL and TTD groups at the same treatment conditions (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). #indicates significant differences of H_2O_2/MitoTEMPO/H_2O_2+MitoTEMPO-exposed groups versus the corresponding basal conditions (##$P < 0.01$; ###$P < 0.001$); ⁺indicates significant differences between CTRL and TTD groups treated with H_2O_2+MitoTEMPO vs only H_2O_2 at the same time point (⁺$P < 0.05$; ⁺⁺⁺$P < 0.001$). Results were analyzed by factorial ANOVA, with *post-hoc* Tukey's multiple comparisons test. CTRL, control subject; TTD, trichothiodystrophy.

Increased lipid peroxidation levels and DNA damage in TTD fibroblasts

After detecting unbalanced oxidants and mtROS production in TTD primary cells, we next explored the effect of H_2O_2-treatment on the levels of lipid peroxidation (LPO) and DNA damage in CTRL and TTD cells. LPO is considered to be involved in the pathogenesis of several disorders such as cancer, atherosclerosis, inflammatory conditions, and neurological diseases (Pecorelli *et al.* 2011, Zhong & Yin 2015, Di Domenico *et al.* 2017, Nègre-Salvayre *et al.* 2017). By using the oxidation-sensitive BODIPY C11 LPO sensor, we could detect the presence of ROS in the cell membranes (green fluorescence). In particular, the levels of the oxidized BODIPY C11 signal raised only after 24h-H_2O_2 exposure in CTRL fibroblasts (Fig. 3A and B). Conversely, TTD cells exhibited constitutive high levels of oxidized

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/

Redox Experimental Medicine (2024) **2024** e240010
https://doi.org/10.1530/REM-24-0010

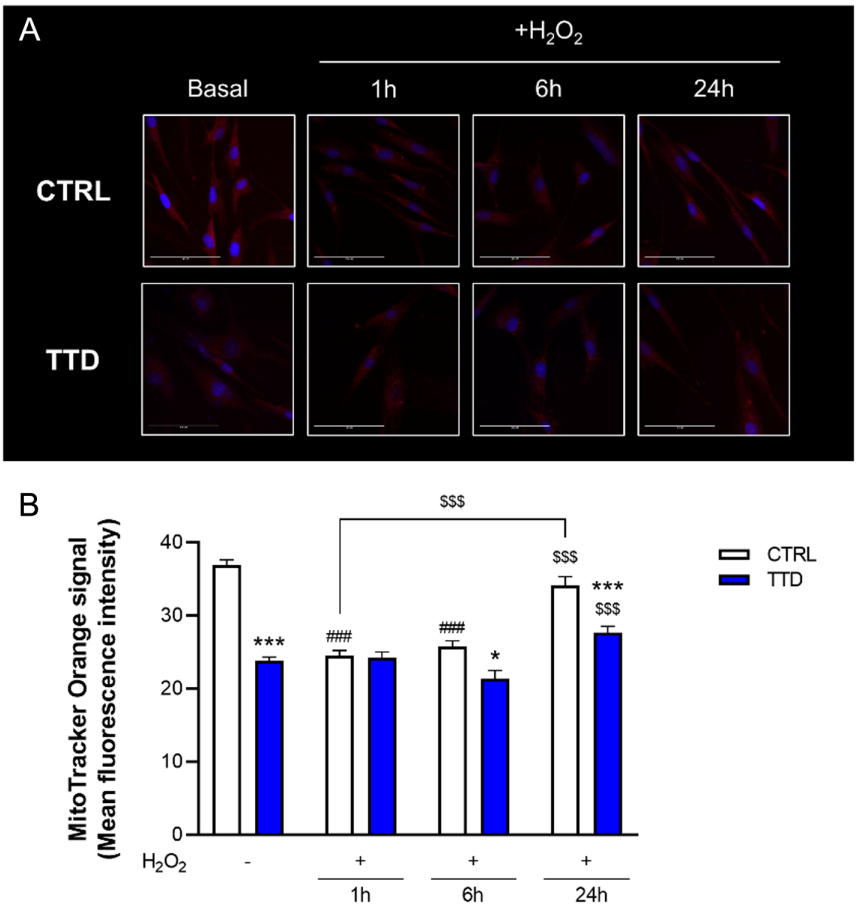

Figure 2

H_2O_2 affects mitochondrial membrane potential differently in CTRL and TTD fibroblasts. (A) Representative images and (B) analysis of MitoTracker Orange fluorescence signal in control and TTD fibroblasts exposed to hydrogen peroxide challenge (100 µM) for 1 h, 6 h and 24 h. Nuclei are counterstained with DAPI. Objective 40×. Bar = 100 µm. Data were expressed as means ± S.E.M., and are the result of three different cultures of cells from n = 1 control subject and n = 1 TTD patient. *indicates significant differences between CTRL and TTD groups at the same treatment conditions (*P < 0.05; ***P < 0.001). #indicates significant differences of H_2O_2-exposed groups versus the corresponding basal conditions (###P < 0.001). $indicates significant differences of H_2O_2-exposed groups versus the previous time-points ($$$$P$ < 0.001). Results were analyzed by factorial ANOVA (with 2 × 2 × 3 design), with *post-hoc* Tukey's multiple comparisons test. CTRL, control subject; TTD, trichothiodystrophy.

BODIPY C11 signal, while upon H_2O_2 treatment the green fluorescence levels were first reduced (1h-treatment vs basal TTD) and then strongly augmented at a longer time-point (24h-treatment vs basal TTD and vs CTRL+H_2O_2 24h) (Fig. 3A and B).

We then analyzed the levels of 4-hydroxynonenal-protein adducts (4-HNE-PAs) in our *ex-vivo* model, since 4-HNE is a highly reactive aldehyde end-product and a biomarker of oxidative stress-induced LPO. It is generated by peroxidation of polyunsaturated fatty acids (PUFAs) in membrane lipid bilayers and its toxicity is mainly due to alterations of cellular functions by the formation of covalent adducts with proteins (Di Domenico *et al.* 2017). As shown in Fig. 3C and D, the levels of 4-HNE-PAs were enhanced in basal TTD cells, as compared to CTRL fibroblasts, which was consistent with BODIPY C11 data (Fig. 3A and B). The H_2O_2 challenge was able to elicit an augment of 4-HNE-PAs levels only in CTRL, but not in TTD cells. Of note, the 4-HNE-PAs signal in TTD fibroblasts, both basal and H_2O_2-stimulated, were similar to those detected in H_2O_2-treated CTRL cells (Fig. 3C and D), suggesting that TTD 4-HNE-PAs were already at maximum levels at baseline.

Then, since ROS and H_2O_2 exposure can also induce DNA double-strand breaks (DSBs) (Li *et al.* 2006), by using fluorescent microscopy, we evaluated the levels of H2AX phosphorylation (Ser 139), as a crucial checkpoint in the recognition and repair of DSBs (Löbrich *et al.* 2010). In CTRL cells, H_2O_2 induced an augment of H2AX phosphorylation after 1h of exposure, followed by a cellular response able to reduce this up-regulation (although not at baseline) (CTRL+H_2O_2 6h and 24h vs basal CTRL). While, H2AX phosphorylation levels were enhanced in TTD cells already in basal condition, showing a further increase upon 1h of H_2O_2 challenge, which remained significantly higher after 6h of exposure (vs basal TTD and vs CTRL+H_2O_2 6h), and decreased only at 24h of treatment. These data suggest that TTD cells have a high steady-state level of DSBs (the enhancement of p-H2AX can be due to other cellular pathways, besides OS (Ivashkevich *et al.* 2012)) and that their DNA repair machinery responds at a slower rate than CTRL cells.

Altered NRF2-BACH1 cross-talk in TTD fibroblasts

In order to elucidate the molecular mechanisms underlying the oxidative damage and the pro-oxidant milieu observed in the TTD *ex-vivo* model, as a first attempt, we investigated the possible involvement of the master regulator of the antioxidant response, nuclear factor erythroid 2-related factor 2 (NRF2). In addition,

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/

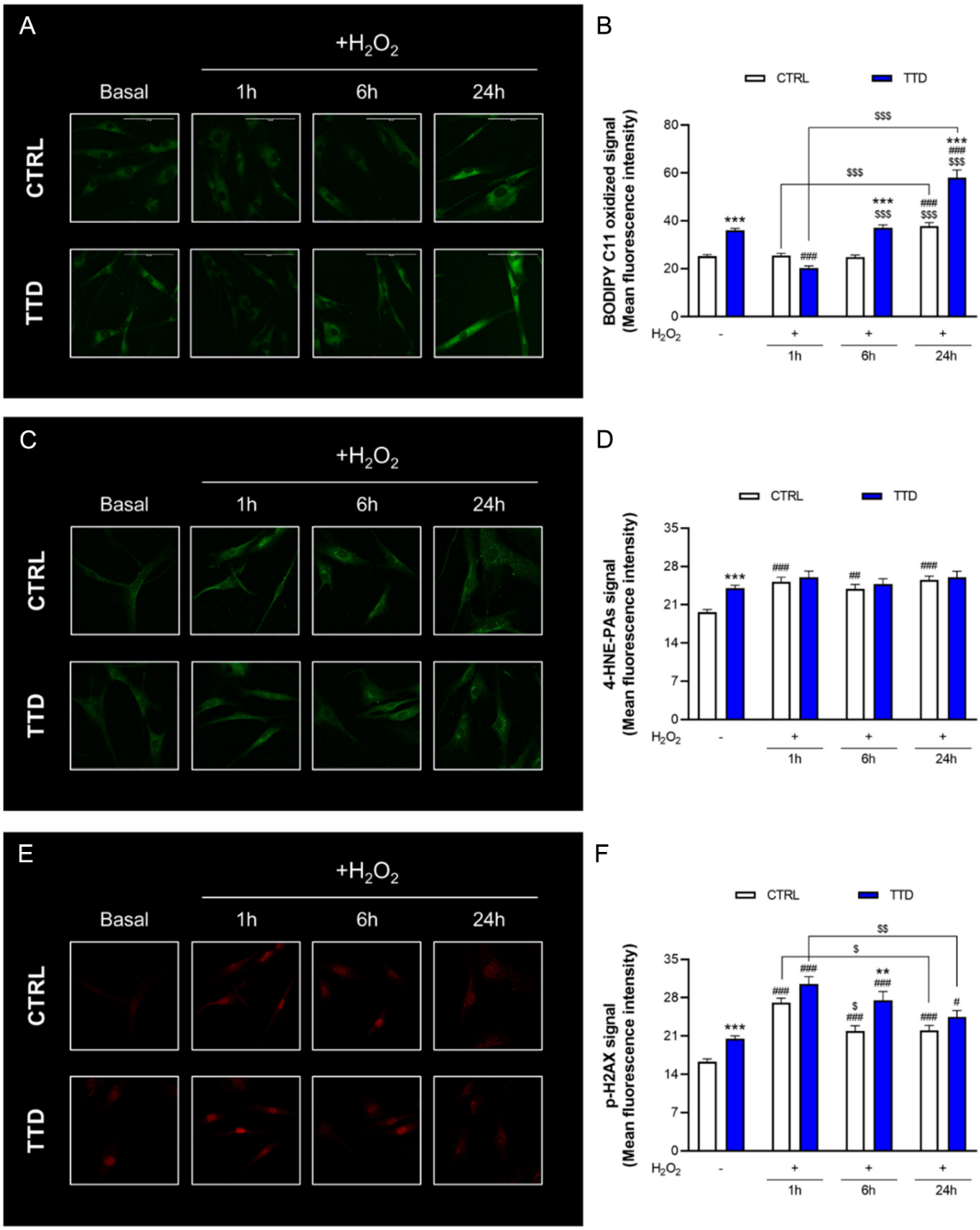

Figure 3

Increased Lipid peroxidation and DNA damage levels in TTD fibroblasts. (A) Representative images and (B) analysis of the immunocytochemical assay with the BODIPY™ 581/591 C11 marker. Peroxidized lipids are shown in green on cells treated with or without hydrogen peroxide (100 µM) for 1h, 6h and 24h. (C and E) Representative images and (D and F) analysis of 4-hydroxynonenal-protein adducts (4-HNE-PAs) and p-H2AX fluorescence levels, detected by IF, in control and TTD fibroblasts, treated as reported above. Objective 40×. Bar = 100 µm. Data were expressed as means ± S.E.M., and are the result of three different cultures of cells from n = 1 control subject and n = 1 TTD patient. *indicates significant differences between CTRL and TTD groups at the same treatment conditions (**P < 0.01; ***P < 0.001). #indicates significant differences of H_2O_2-exposed groups versus the corresponding basal conditions (#P < 0.05; ##P < 0.01; ###P < 0.001). $indicates significant differences of H_2O_2-exposed groups versus the previous time-points ($$P$ < 0.05; $$$P$ < 0.01; $$$$P$ < 0.001). Results were analyzed by factorial ANOVA (with 2 × 2 × 3 design), with *post-hoc* Tukey's multiple comparisons test. CTRL, control subject; TTD, trichothiodystrophy.

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/

we also analyzed the role played by another oxidative stress-responsive transcription factor, BACH1, which by binding to antioxidant response elements (AREs) like NRF2, can prevent the NRF2 binding to the same promoter region, and the expression of genes encoding for antioxidant and detoxification enzymes, as well as cytoprotective proteins (Ahuja *et al.* 2021, Nishizawa *et al.* 2023).

The nuclear translocation of the two transcription factors was evaluated after 1 h of H_2O_2 -/+ MitoTEMPO challenge. As shown in Fig. 4A and C, TTD fibroblasts were characterized by a constitutive decrease of BACH1 nuclear levels, as compared to basal CTRL cells (***P < 0.001). Moreover, CTRL cells exhibited a reduction of BACH1 levels into the nucleus after all the treatments (vs basal CTRL fibroblasts, as indicated

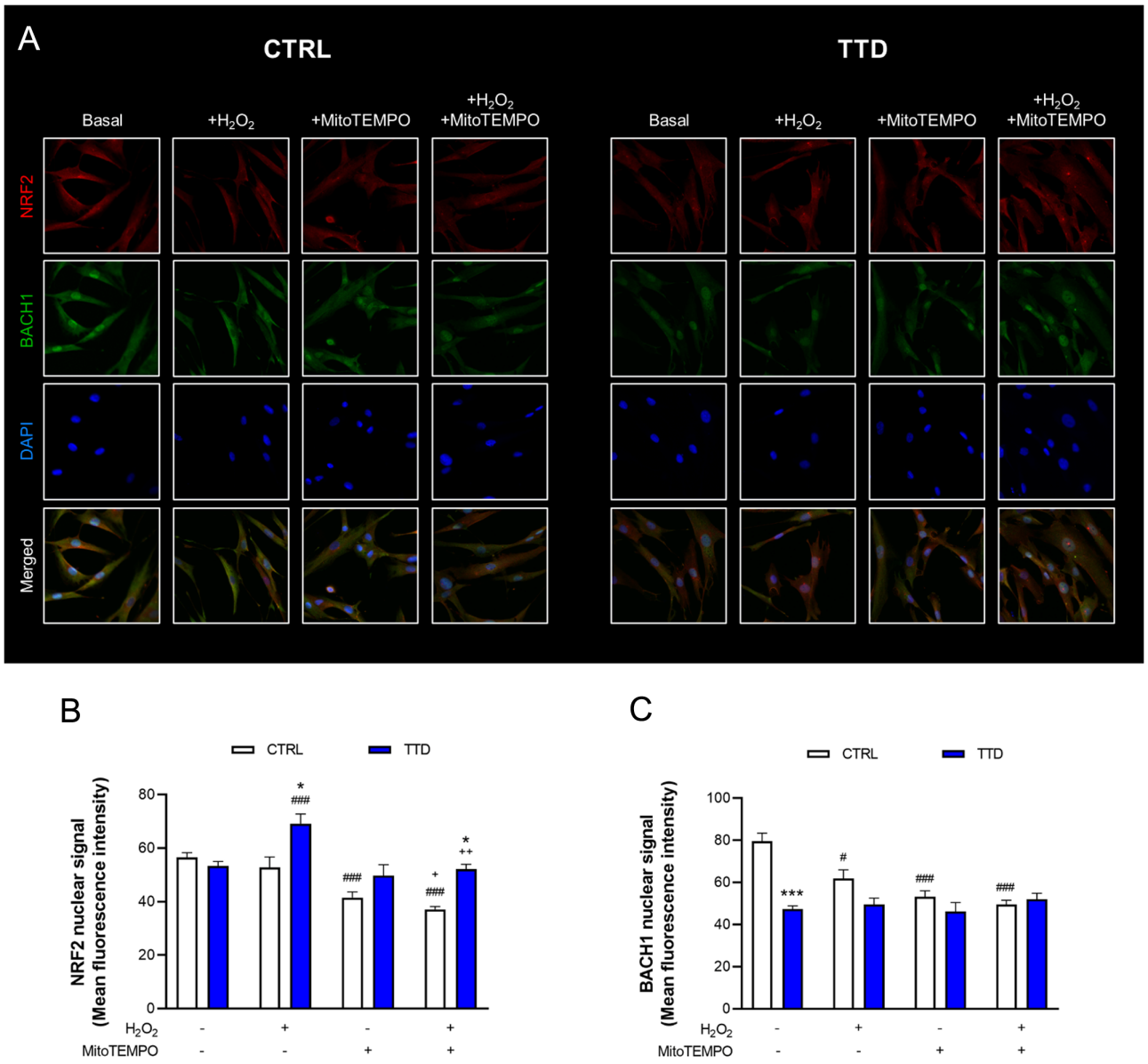

Figure 4

Impaired NRF2–BACH1 interplay in TTD fibroblasts. (A) Representative images and (B and C) analysis of nuclear NRF2 and BACH1 levels, by immunofluorescence, in control and TTD fibroblasts, pre-treated with MitoTEMPO (1 μM for 2 h) and then co-incubated or not with hydrogen peroxide (100 μM) for 1 h. Nuclei are counterstained with DAPI. Objective 40×. Data were expressed as means ± S.E.M., and are the result of three different cultures of cells from n = 1 control subject and n = 1 TTD patient. *indicates significant differences between CTRL and TTD groups at the same treatment conditions (*P < 0.05; ***P < 0.001). #indicates significant differences of H_2O_2/MitoTEMPO/H_2O_2+MitoTEMPO-exposed groups versus the corresponding basal conditions (#P < 0.05; ###P < 0.001); †indicates significant differences between CTRL and TTD groups treated with H_2O_2+MitoTEMPO vs only H_2O_2 (†P < 0.05; ††P < 0.01). Results were analyzed by factorial ANOVA, with *post-hoc* Tukey's multiple comparisons test. CTRL, control subject; TTD, trichothiodystrophy.

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution
4.0 International License.
http://creativecommons.org/licenses/by/4.0/

by #), while in TTD cells BACH1 nuclear signal remained approximately unchanged.

About NRF2, we observed an augmented nuclear localization of this transcription factor in TTD cells after H_2O_2 challenge (vs basal TTD and vs CTRL+H_2O_2) (Fig. 4A and B), maybe due to the combination of exogenous pro-oxidants and endogenous high levels of oxidants, as shown in Fig. 1. While, by scavenging mtROS with MitoTEMPO, we found a significant decrease of nuclear NRF2 signal in both CTRL and TTD fibroblasts exposed to H_2O_2, as compared to the pro-oxidant stimuli alone (Fig. 4A and B), although the NRF2 levels in TTD cells remained higher than CTRL ones (* P <0.05; CTRL+H_2O_2+MitoTEMPO vs TTD+H_2O_2+MitoTEMPO).

Taken together, these results seemed to reveal an altered NRF2–BACH1 interplay in TTD fibroblasts, with an augmented translocation of NRF2 into the nuclear compartment of TTD cells upon pro-oxidant challenge, as a likely consequence of a higher OS, as compared to CTRL cells; whereas presumably, the antioxidant defense system of CTRL fibroblasts were sufficient to cope with the H_2O_2 acute stimulus, without resorting to NRF2 response.

Discussion

Our findings demonstrate, for the first time, the presence of a redox imbalance, associated with an impaired response to pro-oxidant stimuli, in primary skin fibroblasts obtained from a patient with TTD. In particular, our reported results of a constitutive increase of intracellular oxidants and mtROS levels, together with augmented LPO damage at the plasma membrane and proteins (as shown by BODIPY C11 and 4-HNE-PAs levels) seem to indicate a reduced antioxidant defense in TTD cells and suggest that oxidative events are likely to be persistent. In addition, upon a 1 h-pro-oxidant challenge only TTD cells, and not the CTRL ones, induced the translocation of NRF2 into the nuclear compartment, thus likely needing a further antioxidant response to cope with the stress. In this regard, Cho and colleagues found that RNF113A deficiency in HeLa cells was able to trigger the NRF2 pathway, upon an acute treatment (30 min) with H_2O_2 (Cho *et al.* 2024).

Although it is still not clarified whether OS is the cause or the consequence of the phenotypic manifestations typical of TTD, these data add new evidence to the concept of an OS as a key player in both multi-system and genetic pathologies (Perluigi & Butterfield 2012, Cervellati *et al.* 2015, Ohl *et al.* 2016, Vona *et al.* 2021).

We also found that mitochondria are involved in the pro-oxidant milieu of TTD, as shown by the reduced membrane potential and the enhanced levels of mtROS. These latter were reduced to the levels of control cells, by the treatment with MitoTEMPO, a specific scavenger of mitochondrial superoxide anion. This may corroborate the hypothesis of a reduced/malfunctioning antioxidant defense system even at the mitochondrial level. Defective antioxidant response and mitochondrial dysfunctions, known as 'mitochondriopathies', are central hallmarks of several neurologic disorders, genetic pathologies, inflammatory and skin problems (Shulyakova *et al.* 2017, Missiroli *et al.* 2020, Sreedhar *et al.* 2020, Liskova *et al.* 2021).

However, due to the rarity of the pathology, the effects of TTD-related mutations on mitochondrial dysfunctions and OS are poorly studied. Besides the already mentioned paper by Cho and colleagues (Cho *et al.* 2024), another work linked OS to one of the genes involved in TTD – *MARS1* – can be phosphorylated at Ser209 and Ser825 by an extracellular signal-related kinase (ERK1/2) in response to OS, resulting in a reduced specificity for $tRNA^{Met}$ and in more frequent methionine incorporation into newly synthetized proteins, thus increasing cellular reductive capacity (Lee *et al.* 2014, Sung *et al.* 2022). Hence, cells harboring *MARS1* mutations may be more sensitive to oxidative stress, suggesting that these cells respond to OS by promiscuously charging Met to different tRNAs.

It should be anyway mentioned that this study was performed by isolating cells from only one patient as TTD is a very rare disease and it is very difficult to obtain samples. Therefore, before extrapolating these findings to TTD patients more investigations need to be conducted with the aim to confirm our results.

Declaration of interest

G Valacchi is an editorial board member of *Redox Experimental Medicine*. He was not involved in the peer review of this manuscript. The authors declare no competing interests.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

References

Ahuja M, Ammal Kaidery N, Attucks OC, McDade E, Hushpulian DM, Gaisin A, Gaisina I, Ahn YH, Nikulin S, Poloznikov A, *et al.* 2021 Bach1 derepression is neuroprotective in a mouse model of Parkinson's disease. *PNAS* **118** e2111643118. (https://doi.org/10.1073/pnas.2111643118)

Arfin S, Jha NK, Jha SK, Kesari KK, Ruokolainen J, Roychoudhury S, Rathi B & Kumar D 2021 Oxidative stress in cancer cell metabolism. *Antioxidants* **10** 642. (https://doi.org/10.3390/antiox10050642)

Auburger G, Klinkenberg M, Drost J, Marcus K, Morales-Gordo B, Kunz WS, Brandt U, Broccoli V, Reichmann H, Gispert S, *et al.* 2012 Primary skin fibroblasts as a model of Parkinson's disease. *Molecular Neurobiology* **46** 20–27. (https://doi.org/10.1007/s12035-012-8245-1)

Botta E, Theil AF, Raams A, Caligiuri G, Giachetti S, Bione S, Accadia M, Lombardi A, Smith DEC, Mendes MI, *et al.* 2021 Protein instability associated with AARS1 and MARS1 mutations causes

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/

trichothiodystrophy. *Human Molecular Genetics* **30** 1711–1720. (https://doi.org/10.1093/hmg/ddab123)

Cervellati C, Sticozzi C, Romani A, Belmonte G, De Rasmo D, Signorile A, Cervellati F, Milanese C, Mastroberardino PG, Pecorelli A, *et al.* 2015 Impaired enzymatic defensive activity, mitochondrial dysfunction and proteasome activation are involved in RTT cell oxidative damage. *Biochimica et Biophysica Acta* **1852** 2066–2074. (https://doi.org/10.1016/j.bbadis.2015.07.014)

Cho N, Kim Y-E, Lee Y, Choi DW, Park C, Kim J-H, Kim KI & Kim KK 2024 Effect of RNF113A deficiency on oxidative stress-induced NRF2 pathway. *Animal Cells and Systems* **28** 261–271. (https://doi.org/10.1080/19768354.2024.2349758)

Corbett MA, Dudding-Byth T, Crock PA, Botta E, Christie LM, Nardo T, Caligiuri G, Hobson L, Boyle J, Mansour A, *et al.* 2015 A novel X-linked trichothiodystrophy associated with a nonsense mutation in RNF113A. *Journal of Medical Genetics* **52** 269–274. (https://doi.org/10.1136/jmedgenet-2014-102418)

Cordone V, Pecorelli A, Amicarelli F, Hayek J & Valacchi G 2019 The complexity of Rett syndrome models: primary fibroblasts as a disease-in-a-dish reliable approach. *Drug Discovery Today: Disease Models* **31** 11–19. (https://doi.org/10.1016/j.ddmod.2019.11.001)

Cordone V, Ferrara F, Pecorelli A, Guiotto A, Vitale A, Amicarelli F, Cervellati C, Hayek J & Valacchi G 2022 The constitutive activation of TLR4-IRAK1-NFκB axis is involved in the early NLRP3 inflammasome response in peripheral blood mononuclear cells of Rett syndrome patients. *Free Radical Biology and Medicine* **181** 1–13. (https://doi.org/10.1016/j.freeradbiomed.2022.01.017)

Di Domenico F, Tramutola A & Butterfield DA 2017 Role of 4-hydroxy-2-nonenal (HNE) in the pathogenesis of Alzheimer disease and other selected age-related neurodegenerative disorders. *Free Radical Biology and Medicine* **111** 253–261. (https://doi.org/10.1016/j.freeradbiomed.2016.10.490)

Dias V, Junn E & Mouradian MM 2013 The role of oxidative stress in Parkinson's disease. *Journal of Parkinson's Disease* **3** 461–491. (https://doi.org/10.3233/JPD-130230)

Faghri S, Tamura D, Kraemer KH & DiGiovanna JJ 2008 Trichothiodystrophy: a systematic review of 112 published cases characterises a wide spectrum of clinical manifestations. *Journal of Medical Genetics* **45** 609–621. (https://doi.org/10.1136/jmg.2008.058743)

Guo C, Sun L, Chen X & Zhang D 2013 Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regeneration Research* **8** 2003–2014. (https://doi.org/10.3969/j.issn.1673-5374.2013.21.009)

Hayes JD, Dinkova-Kostova AT & Tew KD 2020 Oxidative stress in cancer. *Cancer Cell* **38** 167–197. (https://doi.org/10.1016/j.ccell.2020.06.001)

Itin PH, Sarasin A & Pittelkow MR 2001 Trichothiodystrophy: update on the sulfur-deficient brittle hair syndromes. *Journal of the American Academy of Dermatology* **44** 891–920; quiz 921–924. (https://doi.org/10.1067/mjd.2001.114294)

Ivashkevich A, Redon CE, Nakamura AJ, Martin RF & Martin OA 2012 Use of the γ-H2AX assay to monitor DNA damage and repair in translational cancer research. *Cancer Letters* **327** 123–133. (https://doi.org/10.1016/j.canlet.2011.12.025)

Kholmukhamedov A, Schwartz JM & Lemasters JJ 2013 Isolated mitochondria infusion mitigates ischemia-reperfusion injury of the liver in rats: MitoTracker probes and mitochondrial membrane potential. *Shock* **39** 543. (https://doi.org/10.1097/SHK.0b013e318292300d)

Kleijer WJ, Laugel V, Berneburg M, Nardo T, Fawcett H, Gratchev A, Jaspers NGJ, Sarasin A, Stefanini M & Lehmann AR 2008 Incidence of DNA repair deficiency disorders in Western Europe: xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. *DNA Repair* **7** 744–750. (https://doi.org/10.1016/j.dnarep.2008.01.014)

Kuo ME, Theil AF, Kievit A, Malicdan MC, Introne WJ, Christian T, Verheijen FW, Smith DEC, Mendes MI, Hussaarts-Odijk L, *et al.* 2019 Cysteinyl-tRNA synthetase mutations cause a multi-system, recessive disease that includes microcephaly, developmental delay, and brittle hair and nails. *American Journal of Human Genetics* **104** 520–529. (https://doi.org/10.1016/j.ajhg.2019.01.006)

Kuschal C, Botta E, Orioli D, Digiovanna JJ, Seneca S, Keymolen K, Tamura D, Heller E, Khan SG, Caligiuri G, *et al.* 2016 GTF2E2 mutations destabilize the general transcription factor complex TFIIE in individuals with DNA repair-proficient trichothiodystrophy. *American Journal of Human Genetics* **98** 627–642. (https://doi.org/10.1016/j.ajhg.2016.02.008)

Lee JY, Kim DG, Kim B-G, Yang WS, Hong J, Kang T, Oh YS, Kim KR, Han BW, Hwang BJ, *et al.* 2014 Promiscuous methionyl-tRNA synthetase mediates adaptive mistranslation to protect cells against oxidative stress. *Journal of Cell Science* **127** 4234–4245. (https://doi.org/10.1242/jcs.152470)

Lerner LK, Moreno NC, Rocha CRR, Munford V, Santos V, Soltys DT, Garcia CCM, Sarasin A & Menck CFM 2019 XPD/ERCC2 mutations interfere in cellular responses to oxidative stress. *Mutagenesis* **34** 341–354. (https://doi.org/10.1093/mutage/gez020)

Li Z, Yang J & Huang H 2006 Oxidative stress induces H2AX phosphorylation in human spermatozoa. *FEBS Letters* **580** 6161–6168. (https://doi.org/10.1016/j.febslet.2006.10.016)

Li L, Peng P, Ding N, Jia W, Huang C & Tang Y 2023 Oxidative stress, inflammation, gut dysbiosis: what can polyphenols do in inflammatory bowel disease? *Antioxidants* **12** 967. (https://doi.org/10.3390/antiox12040967)

Liang C, Kraemer KH, Morris A, Schiffmann R, Price VH, Menefee E & DiGiovanna JJ 2005 Characterization of tiger-tail banding and hair shaft abnormalities in trichothiodystrophy. *Journal of the American Academy of Dermatology* **52** 224–232. (https://doi.org/10.1016/j.jaad.2004.09.013)

Liskova A, Samec M, Koklesova L, Kudela E, Kubatka P & Golubnitschaja O 2021 Mitochondriopathies as a clue to systemic disorders—analytical tools and mitigating measures in context of predictive, preventive, and personalized (3P) medicine. *International Journal of Molecular Sciences* **22** 2007. (https://doi.org/10.3390/ijms22042007)

Liu X, Lin J, Zhang H, Khan NU, Zhang J, Tang X, Cao X & Shen L 2022 Oxidative stress in autism spectrum disorder—current progress of mechanisms and biomarkers. *Frontiers in Psychiatry* **13** 813304. (https://doi.org/10.3389/fpsyt.2022.813304)

Löbrich M, Shibata A, Beucher A, Fisher A, Ensminger M, Goodarzi AA, Barton O & Jeggo PA 2010 gammaH2AX foci analysis for monitoring DNA double-strand break repair: strengths, limitations and optimization. *Cell Cycle* **9** 662–669. (https://doi.org/10.4161/cc.9.4.10764)

Maldonado E, Morales-Pison S, Urbina F & Solari A 2023 Aging hallmarks and the role of oxidative stress. *Antioxidants* **12** 651. (https://doi.org/10.3390/antiox12030651)

Markkanen E 2017 Not breathing is not an option: how to deal with oxidative DNA damage. *DNA Repair* **59** 82–105. (https://doi.org/10.1016/j.dnarep.2017.09.007)

Missiroli S, Genovese I, Perrone M, Vezzani B, Vitto VAM & Giorgi C 2020 The role of mitochondria in inflammation: from cancer to neurodegenerative disorders. *Journal of Clinical Medicine* **9** 740. (https://doi.org/10.3390/jcm9030740)

Nakabayashi K, Amann D, Ren Y, Saarialho-Kere U, Avidan N, Gentles S, MacDonald JR, Puffenberger EG, Christiano AM, Martinez-Mir A, *et al.* 2005 Identification of C7orf11 (TTDN1) gene mutations and genetic heterogeneity in nonphotosensitive trichothiodystrophy. *American Journal of Human Genetics* **76** 510–516. (https://doi.org/10.1086/428141)

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/

Nègre-Salvayre A, Garoby-Salom S, Swiader A, Rouahi M, Pucelle M & Salvayre R 2017 Proatherogenic effects of 4-hydroxynonenal. *Free Radical Biology and Medicine* **111** 127–139. (https://doi.org/10.1016/j.freeradbiomed.2016.12.038)

Neikirk K, Marshall AG, Kula B, Smith N, LeBlanc S & Hinton A 2023 MitoTracker: a useful tool in need of better alternatives. *European Journal of Cell Biology* **102** 151371. (https://doi.org/10.1016/j.ejcb.2023.151371)

Nishizawa H, Yamanaka M & Igarashi K 2023 Ferroptosis: regulation by competition between NRF2 and BACH1 and propagation of the death signal. *FEBS Journal* **290** 1688–1704. (https://doi.org/10.1111/febs.16382)

Ohl K, Tenbrock K & Kipp M 2016 Oxidative stress in multiple sclerosis: central and peripheral mode of action. *Experimental Neurology* **277** 58–67. (https://doi.org/10.1016/j.expneurol.2015.11.010)

Orioli D & Stefanini M 2019 Trichothiodystrophy. In *DNA Repair Disorders*. Eds. C Nishigori & K Sugasawa: Singapore: Springer Singapore, pp. 133–159. (https://doi.org/10.1007/978-981-10-6722-8_10)

Palma FR, Gantner BN, Sakiyama MJ, Kayzuka C, Shukla S, Lacchini R, Cunniff B & Bonini MG 2024 ROS production by mitochondria: function or dysfunction? *Oncogene* **43** 295–303. (https://doi.org/10.1038/s41388-023-02907-z)

Pasqui A, Cicaloni V, Tinti L, Guiotto A, Tinti C, Mori A, Bruttini M, Hayek J, Pecorelli A, Salvini L, *et al.* 2024 A proteomic approach to investigate the role of the *MECP2* gene mutation in Rett syndrome redox regulatory pathways. *Archives of Biochemistry and Biophysics* **752** 109860. (https://doi.org/10.1016/j.abb.2023.109860)

Pecorelli A, Ciccoli L, Signorini C, Leoncini S, Giardini A, D'Esposito M, Filosa S, Hayek J, De Felice C & Valacchi G 2011 Increased levels of 4HNE-protein plasma adducts in Rett syndrome. *Clinical Biochemistry* **44** 368–371. (https://doi.org/10.1016/j.clinbiochem.2011.01.007)

Pecorelli A, Belmonte G, Meloni I, Cervellati F, Gardi C, Sticozzi C, De Felice C, Signorini C, Cortelazzo A, Leoncini S, *et al.* 2015 Alteration of serum lipid profile, SRB1 loss, and impaired Nrf2 activation in CDKL5 disorder. *Free Radical Biology and Medicine* **86** 156–165. (https://doi.org/10.1016/j.freeradbiomed.2015.05.010)

Pecorelli A, Ferrara F, Messano N, Cordone V, Schiavone ML, Cervellati F, Woodby B, Cervellati C, Hayek J & Valacchi G 2020*a* Alterations of mitochondrial bioenergetics, dynamics, and morphology support the theory of oxidative damage involvement in autism spectrum disorder. *FASEB Journal* **34** 6521–6538. (https://doi.org/10.1096/fj.201902677R)

Pecorelli A, Cordone V, Messano N, Zhang C, Falone S, Amicarelli F, Hayek J & Valacchi G 2020*b* Altered inflammasome machinery as a key player in the perpetuation of Rett syndrome OxInflammation. *Redox Biology* **28** 101334. (https://doi.org/10.1016/j.redox.2019.101334)

Peoples JN, Saraf A, Ghazal N, Pham TT & Kwong JQ 2019 Mitochondrial dysfunction and oxidative stress in heart disease. *Experimental and Molecular Medicine* **51** 1–13. (https://doi.org/10.1038/s12276-019-0355-7)

Perluigi M & Butterfield DA 2012 Oxidative stress and Down syndrome: a route toward alzheimer-like dementia. *Current Gerontology and Geriatrics Research* **2012** 724904. (https://doi.org/10.1155/2012/724904)

Shafirovich V, Kropachev K, Anderson T, Liu Z, Kolbanovskiy M, Martin BD, Sugden K, Shim Y, Chen X, Min J-H, *et al.* 2016 Base and nucleotide excision repair of oxidatively generated guanine lesions in DNA. *Journal of Biological Chemistry* **291** 5309–5319. (https://doi.org/10.1074/jbc.M115.693218)

Shulyakova N, Andreazza AC, Mills LR & Eubanks JH 2017 Mitochondrial dysfunction in the pathogenesis of Rett syndrome: implications for mitochondria-targeted therapies. *Frontiers in Cellular Neuroscience* **11** 58. (https://doi.org/10.3389/fncel.2017.00058)

Sreedhar A, Aguilera-Aguirre L & Singh KK 2020 Mitochondria in skin health, aging, and disease. *Cell Death and Disease* **11** 444. (https://doi.org/10.1038/s41419-020-2649-z)

Sticozzi C, Belmonte G, Pecorelli A, Cervellati F, Leoncini S, Signorini C, Ciccoli L, De Felice C, Hayek J & Valacchi G 2013 Scavenger receptor B1 post-translational modifications in Rett syndrome. *FEBS Letters* **587** 2199–2204. (https://doi.org/10.1016/j.febslet.2013.05.042)

Sung Y, Yoon I, Han JM & Kim S 2022 Functional and pathologic association of aminoacyl-tRNA synthetases with cancer. *Experimental and Molecular Medicine* **54** 553–566. (https://doi.org/10.1038/s12276-022-00765-5)

Theil AF, Botta E, Raams A, Smith DEC, Mendes MI, Caligiuri G, Giachetti S, Bione S, Carriero R, Liberi G, *et al.* 2019 Bi-allelic TARS mutations are associated with brittle hair phenotype. *American Journal of Human Genetics* **105** 434–440. (https://doi.org/10.1016/j.ajhg.2019.06.017)

Vadarevu H, Juneja R, Lyles Z & Vivero-Escoto JL 2021 Light-activated protoporphyrin IX-based polysilsesquioxane nanoparticles induce Ferroptosis in melanoma cells. *Nanomaterials (Basel, Switzerland)* **11** 2324. (https://doi.org/10.3390/nano11092324)

Vona R, Pallotta L, Cappelletti M, Severi C & Matarrese P 2021 The impact of oxidative stress in human pathology: focus on gastrointestinal disorders. *Antioxidants* **10** 201. (https://doi.org/10.3390/antiox10020201)

Wijnhoven SWP, Beems RB, Roodbergen M, van den Berg J, Lohman PHM, Diderich K, van der Horst GTJ, Vijg J, Hoeijmakers JHJ & van Steeg H 2005 Accelerated aging pathology in ad libitum fed *Xpd*TTD mice is accompanied by features suggestive of caloric restriction. *DNA Repair* **4** 1314–1324. (https://doi.org/10.1016/j.dnarep.2005.07.002)

Zhong H & Yin H 2015 Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria. *Redox Biology* **4** 193–199. (https://doi.org/10.1016/j.redox.2014.12.011)

Zorova LD, Popkov VA, Plotnikov EY, Silachev DN, Pevzner IB, Jankauskas SS, Babenko VA, Zorov SD, Balakireva AV, Juhaszova M, *et al.* 2018 Mitochondrial membrane potential. *Analytical Biochemistry* **552** 50–59. (https://doi.org/10.1016/j.ab.2017.07.009)

Downloaded from Bioscientifica.com at 03/05/2025 12:41:16PM
via Open Access. This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/