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RESEARCH

Decreased hepatic thyroid hormone signaling in systemic and liver-specific but not brain-specific accelerated aging due to DNA repair deficiency in mice

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Abstract

Background: Thyroid hormone signaling is essential for development, metabolism, and response to stress but declines during aging, the cause of which is unknown. DNA damage accumulating with time is a main cause of aging, driving many age-related diseases. Previous studies in normal and premature aging mice, due to defective DNA repair, indicated reduced hepatic thyroid hormone signaling accompanied by decreased type 1 deiodinase (DIO1) and increased DIO3 activities. We investigated whether aging-related changes in deiodinase activity are driven by systemic signals or represent cell- or organ-autonomous changes.

Methods: We quantified liver and plasma thyroid hormone concentrations, deiodinase activities and expression of T3-responsive genes in mice with a global, liver-specific and for comparison brain-specific inactivation of *Xpg*, one of the endonucleases critically involved in multiple DNA repair pathways.

Results: Both in global and liver-specific *Xpg* knockout mice, hepatic DIO1 activity was decreased. Interestingly, hepatic DIO3 activity was increased in global, but not in liver-specific *Xpg* mutants. Selective *Xpg* deficiency and premature aging in the brain did not affect liver or systemic thyroid signaling. Concomitant with DIO1 inhibition, *Xpg*$^{-/-}$ and *Alb-Xpg* mice displayed reduced thyroid hormone-related gene expression changes, correlating with markers of liver damage and cellular senescence.

Conclusions: Our findings suggest that DIO1 activity during aging is predominantly modified in a tissue-autonomous manner driven by organ/cell-intrinsic accumulating DNA damage. The increase in hepatic DIO3 activity during aging largely depends on systemic signals, possibly reflecting the presence of circulating cells rather than activity in hepatocytes.

Keywords

- thyroid hormone
- deiodinase
- aging
- progeria
- DNA damage
- nucleotide excision repair
- liver

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Introduction

Altered neuroendocrine intercellular communication is an important integrative hallmark of aging and is associated with the functional decline in aging (1). In recent years, it has become increasingly clear that thyroid state changes during aging (2, 3, 4, 5). In humans, thyroid-stimulating hormone (TSH) concentrations typically increase with age while concentrations of the bioactive T3 (triiodothyronine) decrease in the elderly population (3, 5). Whether such changes reflect thyroid dysfunction or rather represent an adaptive or even protective response during aging is yet unknown. Thyroid hormones can negatively affect the life span (3, 6, 7). Chronic exposure of excess thyroid hormone leads to a reduced life span (7, 8). Rats rendered hypothyroid have a prolonged life span (9). Snell dwarf mice, which have mutations in the pituitary transcription factor Pit1, resulting in deficiencies in growth hormone (GH), TSH, and prolactin, are for example extremely long-lived (10, 11, 12). Replacement of thyroid hormone in Snell dwarf mice reduces the life span substantially, although their life span remains increased compared with untreated control mice (13). Basically, all long-lived dwarf mutant mice have one or more hormonal deficiencies. However, the endocrine connection with longevity has been primarily investigated for GH and insulin-like growth factor 1 (IGF1) signaling, while the contribution of thyroid hormone signaling is still largely unexplored. Strikingly, compared to mice in which only the GH–IGF1 axis is disrupted (deficient in component(s) of the GH–IGF1 axis), mice with a combination of GH–IGF1 deficiency and thyroid hormone deficiency are among the most extreme longest lived (Fig. 1A). Also, the molecular mechanisms underlying the relationship between changes in thyroid hormone signaling and aging have not been well studied.

Genomic instability, the accumulation of DNA damage throughout life, is another important primary hallmark of aging (1, 14). DNA is constantly challenged by a wide variety of sources of both exogenous (e.g. UV- or X-rays and genotoxic chemicals) and endogenous origin (e.g. reactive oxygen species), but most of the lesions are repaired by dedicated DNA repair processes before they cause cell death or cell malfunction (15, 16). Genetically determined disturbance of DNA repair accelerates the accumulation of DNA damage over time, shortens life span, and drives many age-related diseases and cancer in mammals (17, 18). For instance, *Ercc1*$^{\Delta/-}$ and *Xpg*$^{-/-}$ DNA repair-deficient mice, harboring mutations in key endonucleases excising DNA damage, are both defective in multiple DNA repair pathways, including nucleotide excision repair (NER) and transcription-coupled repair (TCR) and show accelerated aging across many organs and tissues (17, 19, 20). These mouse models of premature aging (also called progeroid mice) can provide important insights into normal aging and are very useful to advance understanding of mechanisms of aging and to explore therapeutic interventions (21, 22). Interestingly, the accelerated aging triggers a protective antiaging 'survival' response, which is similar to the changes observed in long-lived dwarf mice (e.g. Ames and Snell mutants) or mice exposed to dietary restriction (DR), the only well-documented universal anti-aging intervention. This highly intricate response, boosting resilience and defense mechanisms including antioxidant systems at

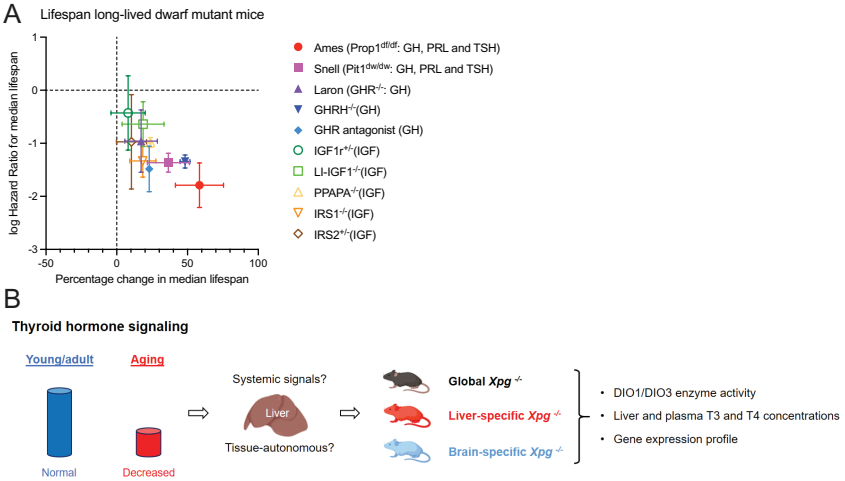

Figure 1
Research question and experimental design. (A) Life span in mice with reduced somato-, lacto-, and/or thyrotropic signaling. The relationship between changes in percentage survival (*x*-axis) and log-hazard ratio effect size (*y*-axis) for median life span. Data were obtained from various life span cohorts (1) and separated by mutation. Mean values ± S.D. of the different cohorts are depicted for the various long-lived dwarf mutant mouse lines. (B) Schematic representation of the research question and experimental design.

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the expense of growth, involves strong suppression of the thyrotropic axis and several other key hormonal axes (20, 23).

Previously, we investigated thyroid hormone signaling in progeroid mice deficient in NER/TCR (24). These results indicated that DNA damage may attenuate thyroid hormone signaling during aging through modulation of deiodinase activity. The hypothyroid state in livers of normal and accelerated aging was associated with decreased activity of thyroid hormone-activating type 1 deiodinase (DIO1) and increased activity of thyroid hormone-inactivating DIO3 (24). It has been reported that, under certain conditions, DIO3 changes in a cell-specific manner without affecting systemic thyroid state (25). At present, it is unclear if aging-related changes in deiodinase activity are driven by systemic signals or represent cell-autonomous changes (Fig. 1B).

To better understand the governance of thyroid hormone signaling, we here investigated mice with a global, liver-specific, and brain-specific inactivation of *Xpg*, one of the key endonucleases critically involved in the NER/TCR processes and when mutated causing three rare, severe (UV-sensitive) human DNA repair syndromes: the highly skin cancer-prone disease xeroderma pigmentosum (XP), the neurodevelopmental accelerated aging disorder Cockayne syndrome (CS), and the dramatic early lethal condition cerebro-oculo-facio-skeletal syndrome (COFS) (19, 26, 27). The global *Xpg* knockout (KO) (*Xpg*$^{-/-}$) mouse mutant is characterized by a shortened life span of about 18 weeks and accelerated the onset of multiple progressive aging features, most pronounced in liver and brain (19, 21, 28). The liver-specific *Alb-Xpg* and brain-specific *Emx-Xpg* KO mice exhibit only severe tissue-specific features of premature aging (19, 29). Our findings suggest that DIO1 activity during aging is predominantly modified in a tissue-autonomous manner driven by organ/cell-intrinsic accumulating DNA damage. The increase in hepatic DIO3 activity during aging largely depends on systemic signals, possibly reflecting the presence of other cells rather than activity in hepatocytes.

Methods summary

The generation and characterization of the different mouse models have been previously described (19). Experiments were performed in accordance with the Principles of Laboratory Animal Care and the guidelines approved by the Dutch Ethical Committee in full accordance with European legislation (permit # 139-12-18).

The activities of the deiodinases DIO1 and DIO3 and concentrations of plasma and liver T3 and T4 were measured as reported previously (30, 31, 32).

Quantification of mRNA of T3-responsive genes was done according to standard procedures.

All statistical analyses were performed using GraphPad Prism (version 9.0.0). Statistical analysis on real-time qPCR data was calculated using dCT values. *P*-values expressed as $*P < 0.05$; $**P < 0.01$, $***P < 0.001$ were considered to be significant. Comparisons for two groups were calculated by unpaired two-tailed Student's *t*-tests.

A full description of the methods is provided in the Supplementary Methods.

Results

We first assessed deiodinase activity levels in livers of full-body *Xpg*$^{-/-}$ DNA repair-deficient progeroid mice. We chose two ages: 4 weeks, when these mice only show minor symptoms of DNA damage accumulation and accelerated aging, and 14 weeks, when the mutant mice exhibit numerous progeroid characteristics, including prominent signs of premature aging in the neuronal system and liver, without being moribund (19, 21, 28). Measuring DIO1 activity at 4 weeks of age showed a minor trend of reduced activity (Fig. 2A). At the age of 14 weeks, DIO1 activities in *Xpg*$^{-/-}$ liver were >6-fold ($P = 0.0003$) lower compared to wild-type (Wt) littermates (Fig. 2A). DIO3 activity in liver of *Xpg*$^{-/-}$ mice changed in the opposite manner (Fig. 2B; ~2-fold increase at 14 weeks, $P = 0.041$). The pattern of decreased DIO1 and increased DIO3 activity, with more pronounced effects in older animals, was reminiscent of the changes previously observed in other accelerated aging models (24), suggesting that *Xpg*$^{-/-}$ mice are relevant for exploring changes in thyroid state during aging.

To investigate if these deiodinase changes are tissue autonomous or not, we next assessed these measurements in *Xpg*-mutant mice harboring the genetic defect only in liver (under the *albumin*-Cre promoter specific to hepatocytes (33); hereafter named *Alb-Xpg*) (19, 29). We employed (fore)brain-specific (under the *Emx1*-Cre promoter specific to neuronal progenitor cells (34); see Supplementary Methods) deletion of *Xpg* (hereafter named *Emx-Xpg*) (19) as a negative control, as brain-specific accelerated aging is not expected to

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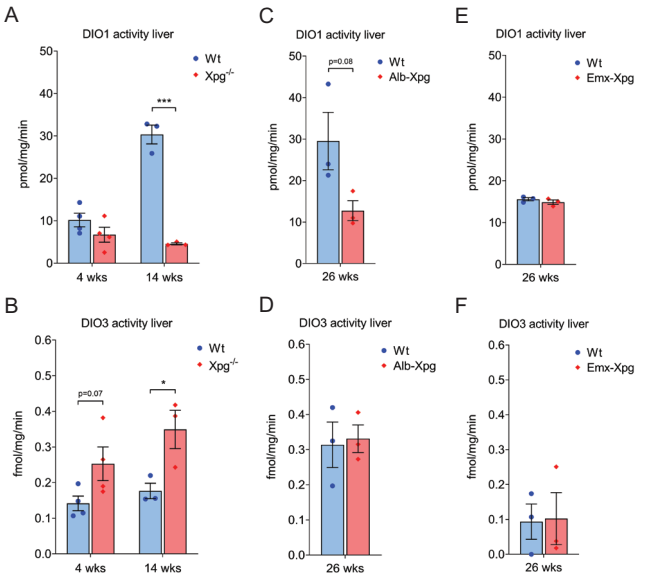

Figure 2
Deiodinase activity in liver. Deiodinase 1 (DIO1) and 3 (DIO3) activity in livers of DNA repair-deficient (indicated in red) 4- and 14-week-old male *Xpg*$^{-/-}$ mice (A, B), 26-week-old liver-specific male *Alb-Xpg* mice (C, D) and 26-week-old brain-specific male and female *Emx-Xpg* mice (E, F). $n = 3–4$ animals/group. Wild-type (Wt) littermate controls are indicated in blue. Error bars denote mean ± s.e. $^{*}P < 0.05$, $^{***}P < 0.001$.

affect liver thyroid hormone signaling. Both organs are known to display many progressive features of premature aging in short-lived full-body *Xpg*$^{-/-}$ mutants (19, 21, 28). Similarly, the tissue-specific mice show prominent local premature aging features from half a year of age onward, without causing early death (19). We therefore assessed thyroid state changes in both tissue-specific mutants at 26 weeks of age. In 26-week-old *Alb-Xpg* mice, when accelerated aging signs are clearly present in only liver (19, 29), liver DIO1 activity appeared decreased, like in *Xpg*$^{-/-}$ mice, while DIO3 activity was similar to that of controls (Fig. 2C and D). In livers of *Emx-Xpg* mice we did not find changes in DIO1 and DIO3 activities (Fig. 2E and F), arguing against systemic changes in the thyroid hormonal axis as a result of brain-specific premature neurodegeneration.

To find out whether the changes in enzyme activity were controlled at the level of gene expression or posttranscriptionally we determined the mRNA abundance by qRT-PCR. The observed changes in deiodinase activities in livers of full-body *Xpg*$^{-/-}$ mice and liver-specific *Alb-Xpg* mice were mirrored by alterations in *Dio1* and *Dio3* mRNA levels (Fig. 3A, B, and C), indicating that the changes are regulated at least in part transcriptionally.

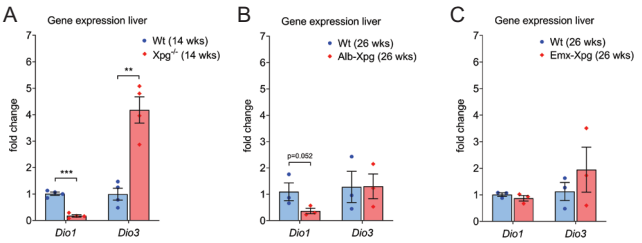

Figure 3
Deiodinase gene expression in liver. *Dio1* and *Dio3* gene expression in livers of 4- and 14-week-old male *Xpg*$^{-/-}$ mice (A), 26-week-old liver-specific male *Alb-Xpg* mice (B), and 26-week-old brain-specific male and female *Emx-Xpg* mice (C). $n = 3–4$ animals/group. Error bars denote mean ± s.e. $^{**}P < 0.01$, $^{***}P < 0.001$.

Next, we measured thyroid hormone concentrations in both livers and plasma in all three animal models and Wt controls (Fig. 4). Liver T3 concentrations were only decreased in full-body *Xpg*$^{-/-}$ mice (Fig. 4A) but not in tissue-specific *Alb-Xpg* or *Emx-Xpg* mice (Fig. 4B and C). No changes in liver T4 concentrations were seen (Fig. 4D, E, and F). Overall, decreased plasma T3 and T4 concentrations were noted in aged *Xpg*$^{-/-}$ mice (Fig. 4G and J), while those values were increased in young *Xpg*$^{-/-}$ and in tissue-specific *Alb-Xpg* mice (Fig. 4H and K). No changes in plasma T3 and T4 concentrations were observed in *Emx-Xpg* mice (Fig. 4I and L), indicating that accelerated neurological aging in forebrain does not cause systemic changes in the thyroid hormonal system.

To explore the net biological effect of the abovementioned changes in hormone concentrations and deiodinase activities, we quantified expression levels of a panel of genes that have been shown to be regulated by thyroid state (24). Consistent with the lower T3 concentrations, the decreased DIO1 and increased DIO3 activities, many thyroid hormone responsive genes were concordantly downregulated in livers of *Xpg*$^{-/-}$ mice, with changes being more pronounced in older animals (Fig. 5A and B). While *Alb-Xpg* mice showed a similar tendency for downregulation (Fig. 5C), no consistent changes in this panel of thyroid hormone-responsive genes were seen in *Emx-Xpg* mice compared to Wt control mice (Fig. 5D). We noted a paradox between the elevated circulating T3 and T4 concentrations, but subtle decreased expression of thyroid hormone responsive genes in liver of *Alb-Xpg* mice (Fig. 4H and K vs Fig. 5C). Therefore, we quantified hepatic expression of thyroid hormone-binding proteins for all animal models (Fig. 5E, F, G, and H), clearly showing a 3× upregulation of the high-affinity binding protein *Tbg* in *Alb-Xpg* mice (Fig. 5G), likely explaining the rise of total T3 and T4 in plasma.

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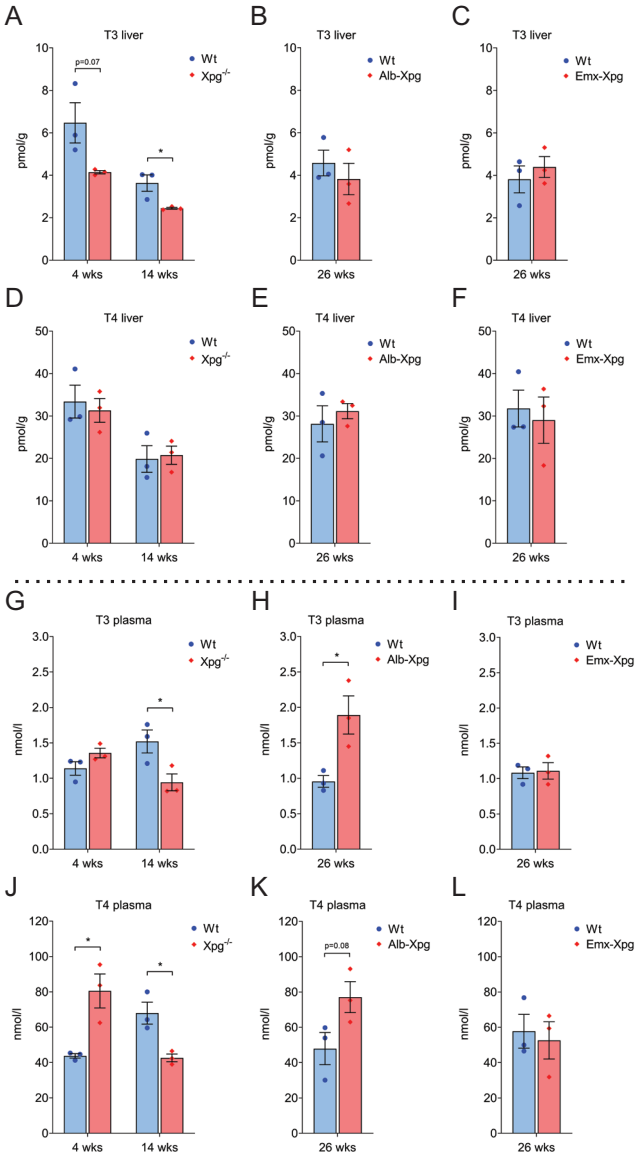

Figure 4
Thyroid hormone concentrations in liver and plasma. Liver T3 and T4 concentrations in 4- and 14-week-old male and female *Xpg*$^{-/-}$ mice (A, D), 26-week-old liver-specific male *Alb-Xpg* mice (B, E), and 26-week-old brain-specific male and female *Emx-Xpg* mice (C, F). Plasma T3 and T4 concentrations in 4- and 14-week-old male and female *Xpg*$^{-/-}$ mice (G, J), 26-week-old liver-specific male *Alb-Xpg* mice (H, K), and 26-week-old male and female brain-specific *Emx-Xpg* mice (I, L). $n = 3$ animals/group. Error bars denote mean ± s.e. $^*P < 0.05$.

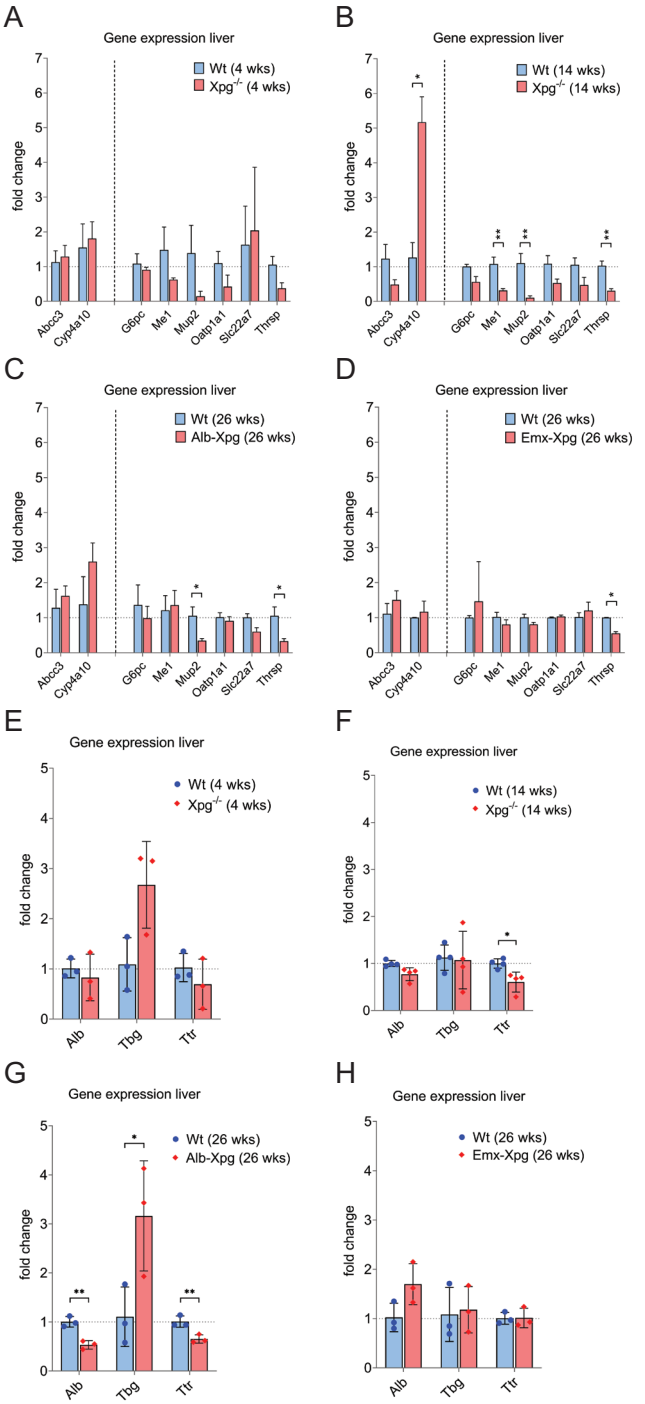

Figure 5
Expression of thyroid hormone responsive genes and thyroid hormone-binding proteins in liver. Gene expression in livers of 4- and 14-week-old male *Xpg*$^{-/-}$ mice (A, B), 26-week-old liver-specific male *Alb-Xpg* mice (C), and 26-week-old brain-specific male and female *Emx-Xpg* mice (D). $n = 3$–4 animals/group. The dotted line separates genes that are upregulated (left) or downregulated (right) in hypothyroidism. *Alb*, *Tbg*, and *Ttr* gene expression in livers of 4-week-old male *Xpg*$^{-/-}$ mice (E), 14-week-old male *Xpg*$^{-/-}$ mice (F), 26-week-old male *Alb-Xpg* mice (G) and 26-week-old male and female *Emx-Xpg* mice (H). $n = 3$–4 animals/group. Error bars denote mean ± s.e. $^*P < 0.05$, $^{**}P < 0.01$.

As hepatic thyroid hormone levels and DIO3 activity differed between *Xpg*$^{-/-}$ and *Alb-Xpg* mice, we tested if differences in the degree of liver damage could be an explanation. At the histological level, both *Xpg*$^{-/-}$ and *Alb-Xpg* mice showed severe signs of liver aging such as hepatocyte polyploidization, seemingly to a greater extent in the *Alb-Xpg* mice (Supplementary Fig. 1A and B, see section on supplementary materials given at

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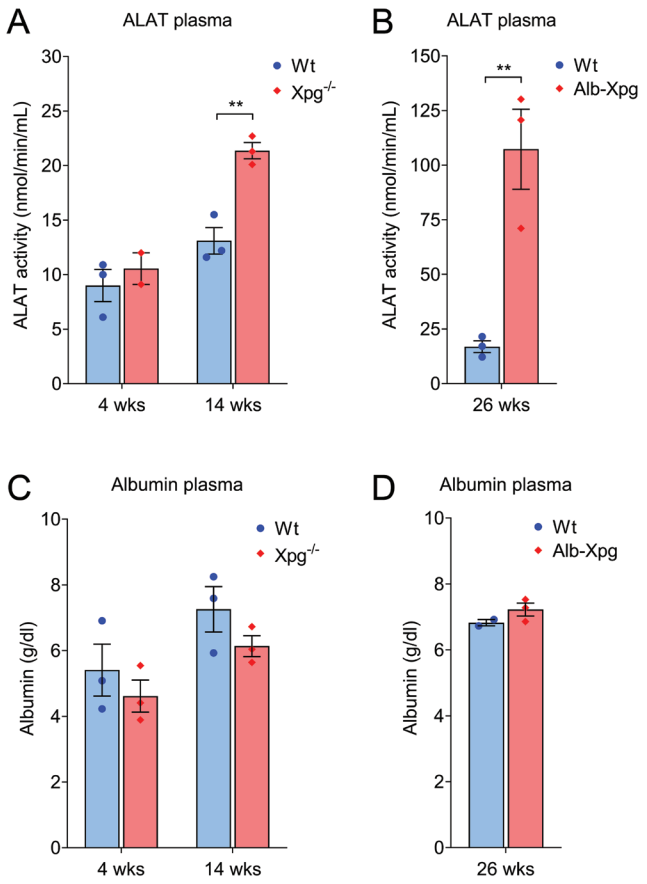

Figure 6
Alanine transferase and albumin concentrations in plasma. Alanine amino transferase (ALAT) concentration in plasma of 4- and 14-week-old male *Xpg*$^{-/-}$ mice (A), and 26-week-old liver-specific male *Alb-Xpg* mice (B). Albumin concentration in plasma of 4- and 14-week-old male *Xpg*$^{-/-}$ mice (C), and 26-week-old, liver-specific male *Alb-Xpg* mice (D). $n = 2$–3 animals/group. Error bars denote mean ± S.E. $^{**}P < 0.01$.

the end of this article) (19). Alanine aminotransferase (ALAT) concentrations as a marker for liver damage were elevated in both animal models and even more pronounced in *Alb-Xpg* mice (Fig. 6A and B). In contrast, liver function appeared normal using albumin production as a proxy marker (Fig. 6C and D).

The noted elevated levels of hepatocyte polyploidization in *Xpg*$^{-/-}$ and *Alb-Xpg* mice could be a sign of cellular senescence (35), which increases during aging and was previously observed in various DNA repair-deficient accelerated aging mouse models (21, 29, 36, 37, 38, 39). Based on the observation that IL-6, a senescence associated factor, could modify deiodinase activity (2, 40, 41), we aimed to measure cellular senescence markers by gene expression as a potential discriminator between global systemic and local cell-autonomous changes. Various expression markers previously identified for the detection of senescence (36, 42) were increased in *Xpg*$^{-/-}$ and *Alb-Xpg* mice (Fig. 7A and B), with none significantly changed in *Emx-Xpg* mice (Fig. 7C), correlating with the degree of polyploidization (Supplementary Fig. 1) and DIO1 inhibition (Fig. 2 and 3) in a cell-autonomous manner. In summary, we observed a reduction in DIO1 activity and related thyroid hormone-responsive genes in conditions of liver damage, while increased DIO3 activity was only noted in livers of global *Xpg*-deficient mice.

Discussion

The present data indicate that hepatic thyroid hormone signaling is changed during aging via tissue-autonomous and nonautonomous ways. Liver-specific and full-body inactivation of the DNA repair gene *Xpg,* causing local or body-wide accelerated aging, results in decreased DIO1 expression and activity, while an increased DIO3 expression and activity is only present when DNA repair is globally hampered. As anticipated, *Xpg* deficiency and accelerated aging in brain does not exert any effect on thyroid signaling in liver.

To obtain a better mechanistic understanding of changes in thyroid hormone signaling during aging, model organisms can be used. Premature aging mice with segmental *bona fide* features of normal aging, reflect valuable models to study the normal aging process (17, 19, 21, 29, 43, 44, 45). Previously, we investigated two different prematurely aged DNA

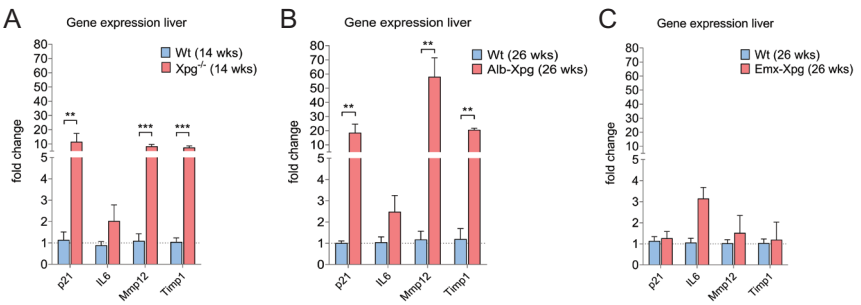

Figure 7
Expression of senescence-associated factors in liver. *P21*, *IL-6*, *Mmp12*, and *Timp1* expression in livers of 14-week-old male *Xpg*$^{-/-}$ mice (A), 26-week-old liver-specific male *Alb-Xpg* mice (B), and 26-week-old brain-specific male and female *Emx-Xpg* mice (C). $n = 3$–4 animals/group. Error bars denote mean ± S.E. $^{**}P < 0.01$, $^{***}P < 0.001$.

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repair-deficient animal models and Wt aging mice (24). The large similarity in changes in thyroid hormone regulation between premature and normal aging indicated the usefulness of premature aging models in this field (24).

Here, we studied mice deficient in *Xpg* as another model for aging (19, 21). A principal finding was that we observed a hypothyroid state in liver accompanied by decreased DIO1 and increased DIO3 activity. The consistency with similar findings in other models supports the robustness of the observations (24). However, in mice with global inactivation of DNA repair it cannot be distinguished to which extent the changes in hepatic deiodinase activities result from systemic signals or from cell-autonomous processes. Therefore, the present study was designed to address this research question. In both full-body and liver-specific *Xpg* KO mice, DIO1 activity and expression was decreased consistent with reduced liver T3 concentrations. In contrast, DIO3 activity and expression was specifically increased in full-body *Xpg*$^{-/-}$ mice, and not subjected to changes in liver-specific *Alb-Xpg* mice. Interestingly, even though several features of hepatic accelerated aging in 26-week-old liver-specific mutants appeared more severe compared to the 14-week-old full-body *Xpg* mutant mice, the magnitude of DIO1 changes were more moderate, implying that changes in DIO1 are not solely explained by liver-autonomous phenomena. Brain-specific depletion of *Xpg* did not result in any significant changes in liver DIO1 and DIO3 activity or T3 and T4 levels.

The observed changes in DIO3 however rather seem to originate from systemic factors as indicated by the absence of DIO3 elevation in both tissue-specific mutants. However, the age of the *Xpg*$^{-/-}$ and *Alb-Xpg* mice investigated are not identical as the rate of aging differs between systemic and local DNA repair deficient mutants (19, 46). Also, we cannot fully rule out a contribution of aging Kupfer cells or other liver cell-types in DIO3 activation as in the *Alb-Xpg* mice only hepatocytes are affected and thus could, when too severely damaged at later ages, be repopulated via yet unaffected liver stem cells, in contrast to *Xpg*$^{-/-}$ mice ((19) and unpublished observations). In line with previous observations, the activation of DIO3 in our study could originate from infiltrating cells (e.g. neutrophils or macrophages) invading the aged liver (47). Alternatively, metabolic factors could, in several cell-types including hepatocytes, elevate DIO3 expression and activity (48), which are components affected more in *Xpg*$^{-/-}$ over *Alb-Xpg* mice (19, 49). It remains however to be identified which cell types or conditions are truly responsible for the increased expression and activity of DIO3. With the current wisdom that DIO3 can be re-activated under certain conditions, future studies should explore whether tissue DIO3 activity reflects endogenous cells or invading cells.

Both global *Xpg*$^{-/-}$ mice and liver-specific *Alb-Xpg* mice show elevated levels of cellular senescence markers in liver, such as hepatocyte polyploidization, p21 expression and senescence-associated-secretory-phenotype (SASP) activation, of which at least IL-6 has been implicated in DIO3 regulation before. Should this be a causal link between aging, senescence and thyroid hormonal state, it can be speculated that activity of deiodinases may be downstream of inflammation pathways that are changed upon aging (2, 40). SASP activation is a prominent factor that accumulates with aging and upon excessive DNA damage occurring e.g. after chemotherapy (2, 36, 50, 51, 52). In this scenario the tissue-autonomous thyroid hormonal changes might be part of a broad 'survival' response to (accelerated) aging, driven at least in part by accumulation of DNA lesions. This response attempts to counteract the accelerated aging by boosting resilience mechanisms (e.g. antioxidant defenses) and reducing metabolism and growth (IGF1/GH) and strongly resembles the anti-aging response triggered by DR (20, 23, 53, 54). Since we found that DNA repair-deficient progeroid mouse models, including *Xpg*$^{-/-}$ mice respond remarkably well to the anti-aging effects of DR (21), the reduced thyroid hormonal activity in this mutant may be an important component of this protective 'survival' response. Indeed, the response elicited by DR also encompasses attenuation of the thyroid hormonal axis (55, 56, 57, 58).

Together, these findings suggest that DIO1 activity during liver-aging is predominantly reduced in a tissue-autonomous manner, while the increased DIO3 activity during aging may largely depend on circulating cells infiltrating the liver.

We realize our study has several limitations. First, the *Xpg* deficient mouse models used in the present study represent models of segmental accelerated aging. Although they display an extremely broad variety of symptoms and pathologies also observed in normal mouse and human aging (17, 21, 44), we cannot exclude specific features being more pronounced or lacking. Second, the hormone measurements in both tissue and plasma are total hormone concentrations, while only the

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free hormone fraction is available for biological action. Indeed, the thyroid hormone binding protein Tbg, which carries the majority of circulating T3 and T4, was elevated in *Alb-Xpg* mice, potentially explaining why thyroid-responsive genes were downregulated, presumably following lower free hormone concentrations, while the total T3 plasma concentrations are elevated. Third, our study did not address which cells in the liver express DIO3. Our attempts so far were yet inconclusive but point to potential involvement of neutrophils or macrophages as noted before (47). Should that be the case, the function of increased DIO3 in these cells during aging remains to be elucidated.

Whether the changes in deiodinases during aging are beneficial cannot be established at this stage. In the context of many metabolic processes being reduced in aging, a further reduction of thyroid state might have beneficial effects (6, 7). Indeed, observational studies in older individuals may hint at increasing healthy lifespan in elderly with a lower thyroid state (59). The interplay between genetic, metabolic and environmental factors determines a unique thyroid biography in individuals. (2, 60). Lastly, interventional studies including DR in premature aging mice indicate the value of such models to explore therapeutic strategies for extending healthy lifespan (21, 22). Future studies should investigate to which extent the thyrotrophic axis influences the rate of aging, age-related multi-morbidity and lifespan in order to see whether and how this key hormonal axis can be exploited to promote healthy aging.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/ETJ-22-0231.

Declaration of interest

The authors declare that there is no conflict of interest that could prejudice the impartiality of the research reported. Edward Visser is on the editorial board of *European Thyroid Journal*. Edward Visser was not involved in the review or editorial process for this paper, on which he is listed as an author.

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Author contribution statement

SB, JHJH, WPV, and WEV designed studies and wrote the manuscript. SB, RvH, and SL performed the experiments. MM, RPP, and VMD provided intellectual input.

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