Expression of β -1,4-galactosyltransferases during Aging in *Caenorhabditis elegans*

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Keywords

bre-4 · sqv-3 · Lifespan regulation · Glycosylation · Biomarker

Abstract

Background: Altered plasma activity of β -1,4-galactosyltransferases (B4GALTs) is a novel candidate biomarker of human aging. B4GALT1 is assumed to be largely responsible for this activity increase, but how it modulates the aging process is unclear at present. **Objectives:** To determine how expression of B4GALT1 and other B4GALT enzymes changes during aging of an experimentally tractable model organism, *Caenorhabditis elegans*. **Methods:** Targeted analysis of mRNA levels of all 3 *C. elegans* B4GALT family members was performed by qPCR in wild-type and in long-lived *daf-2* (insulin/IGF1-like receptor)-deficient or germline-deficient animals. **Results:** *bre-4* (B4GALT1/2/3/4) is the only B4GALT whose expression increases during aging in wild-type worms. In addition, *bre-4* levels also rise during aging in long-lived *daf-2*-deficient worms, but not in animals that are long-lived due to the lack of germline stem cells. On the other hand, expression of *sqv-3* (B4GALT7) and of *W02B12.11* (B4GALT5/6) appears decreased or constant, respectively, in

all backgrounds during aging. **Conclusions:** The age-dependent *bre-4* mRNA increase in *C. elegans* parallels the age-dependent B4GALT activity increase in humans and is consistent with *C. elegans* being a suitable experimental organism to define potentially conserved roles of B4GALT1 during aging.

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Introduction

Aging is a major and common risk factor for many severe human diseases such as cardiovascular diseases, neurodegeneration, and cancer [1]. However, as individuals age at different rates, there is a great need for markers that, alone or in combination, can predict susceptibility to disease and death more reliably than chronological age [1–3]. Examples of candidate molecular biomarkers of aging include DNA methylation status, plasma levels of certain cytokines, hormones and lipids, and altered gly-

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cosylation of particular proteins such as immunoglobulin G (IgG) [3–5]. A recent study also proposed increased plasmatic activity of β -1,4-galactosyltransferases (B4GALTs) as a novel biomarker of aging [6]. Even though the B4GALTs involved were not formally determined, B4GALT1 is assumed to be the major mediator of this increase [6].

B4GALTs are a class of evolutionarily conserved enzymes that catalyze the addition of a galactose (Gal) sugar moiety from a common UDP- α -Gal donor to different oligosaccharide acceptors via a β -1,4-glycosidic bond. Given the diverse signaling and structural functions of their products, B4GALTs modulate a broad variety of biological processes, such as protein folding, cell-cell interactions, and immune defense [7]. Among the 7 B4GALT enzymes in humans, B4GALT1–6 contribute to the synthesis of glycoproteins and glycolipids by transferring Gal to N-acetylglucosamine (GlcNAc) residues at the nonreducing ends of oligosaccharide chains, while B4GALT7 participates in the synthesis of the oligosaccharide linker in proteoglycans by transferring Gal to a Xylose (Xyl) moiety [7, 8]. All B4GALTs are type-II transmembrane proteins located in the Golgi or transgolgi network, with their catalytic domains facing the lumen [7, 8]. In addition, B4GALT1 can function as a matrix receptor on the cell surface [9]. Moreover, a soluble isoform of B4GALT1 is expressed in mammary tissue and constitutes the catalytic subunit of lactose synthase, which adds Gal to a free Glucose (Glc) molecule [7, 8]. In humans, increased levels of B4GALT1 have been associated with both, beneficial and detrimental prognosis or outcome in various types of cancer. Examples include acute myeloid leukemia, chronic myeloid leukemia [10], nonmetastatic clear-cell renal-cell carcinoma [11], and muscle-invasive bladder cancer [12], where its elevated expression in bone marrow mononuclear cells or tumor tissue correlated with chemoresistance or decreased overall survival [10–12]. On the other hand, in seminoma patients, it was found that high expression of B4GALT1 in peripheral T lymphocytes is associated with superior tumor control [13].

Studies on invertebrate model organisms such as the roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* provided key insights into the biology of lifespan regulation that apparently also apply to human aging [14]. A prototypic example is provided by reduced insulin/insulin-like growth factor (IGF1)-like signaling (IIS), which was first observed in *C. elegans* to induce a dramatic, approximately two-fold increase in lifespan, and was subsequently shown to promote longevity in mammals [15–17]. In *C. elegans*, lifespan extension

by reduced IIS, e.g., due to a reduction of function mutations in the gene *daf-2*, which encodes the common ortholog of the mammalian insulin- and IGF1 receptors, is dependent on *daf-16*, an ortholog of mammalian FOXO transcription factors [14]. Of note, genetic variants in *FOXO3* have been repeatedly and robustly associated with lifespan in humans [18]. Beyond the *daf-2* mutation, additional mechanisms for lifespan extension have been described in *C. elegans*, e.g., the ablation of germline stem cells (GSCs) through laser microsurgery or through temperature-sensitive mutations in the Notch-receptor ortholog *glp-1* [19, 20]. Interestingly, this GSC-regulated longevity pathway also requires *daf-16*, but it appears to not engage *daf-2* [14, 20].

The *C. elegans* genome encodes 3 members of the B4GALT family, BRE-4, SQV-3, and W02B12.11, but only the first 2 family members have already been functionally characterized. *Bre-4* was identified in a genetic screen for *Bacillus thuringiensis* pore-forming toxin-resistant mutants [21]. In wild-type animals, BRE-4 confers sensitivity to these toxins through its action in intestinal cells, where it contributes to the glycosylation of membrane lipids, which can then act as toxin receptors [22, 23]. In addition, *bre-4* positively regulates the Notch ortholog *lin-12* during certain cell fate decisions [24], raising the possibility that it can mediate protein glycosylation, not just in vitro [25], but also in vivo. Lastly, *bre-4* is required for proper developmentally timed sleep, although the underlying mechanism here has not been determined [26]. On the other hand, *sqv-3* was initially isolated in a mutagenesis screen based on its squashed vulva phenotype [27]. Beyond normal vulval development, *sqv-3* is required for normal oogenesis and embryonic development, apparently via its function in the synthesis of extracellular matrix proteoglycans [28].

Whether the observed increase in human plasma B4GALT activity represents a cause or a consequence of aging is unclear at present. On the other hand, the conservation of B4GALT genes between humans and *C. elegans* raises the possibility that they also share some common physiological activities, and that a potentially conserved role for B4GALT in lifespan regulation can be defined by studies on *C. elegans*. This hypothesis implies that B4GALT activity also increases during *C. elegans* aging. A potential and easily measurable source of increased B4GALT activity would be elevated mRNA levels. In this study, we examined mRNA expression of the B4GALT1 ortholog *bre-4*, and of the 2 other B4GALT genes, *sqv-3* and *W02B12.11*, during aging in wild-type *C. elegans* as well as in 2 long-lived *C. elegans* mutants, *daf-2* and *glp-1*.

Fig. 1. mRNA levels of *bre-4*, but not of other *C. elegans* B4GALT family members, change during aging. **a** Evolutionary relationship between B4GALT enzyme family members in *C. elegans* and humans. *C. elegans* enzymes are colored in light grey, human enzymes in dark grey. Numbers indicate % identity and % similarity of the respective human protein to *C. elegans* BRE-4 (B4GALT1/2/3/4), W02B12.11 (B4GALT5/6), or SQV-3 (B4GALT7). **b** mRNA levels of the 3 B4GALTs were measured by qPCR and normalized to housekeeping genes in the strains indicated on day 1, day 5, and day 10 of adulthood. Data represent the mean expression relative to day 1 $\pm$ SD from 4 biological replicates. Statistical significance was determined by two-way ANOVA with Bonferroni post hoc tests (***) $p < 0.001$.

Our observation of *bre-4* mRNA levels increasing during aging in wild-type *C. elegans* is reminiscent of the elevated B4GALT activity reported in elderly humans, thus providing the first evidence of *C. elegans* being a suitable model system to study potentially conserved roles for B4GALTs during aging.

Materials and Methods

Strains and Culture

The following strains/genotypes were analyzed in this study: N2E: wild-type; CF3942: *glp-1(e2144ts)* III (from CF1903 [29, 30], outcrossed 12 $\times$ to N2E); CF4087: *daf-2(e1370)* III (from CF1041 [31], outcrossed 12 $\times$ to N2E). Worms were cultured following standard protocols on NG agar plates seeded with *E. coli* OP50 [32]. Worms carrying the *glp-1(e2144ts)* mutation and corresponding *glp-1(+)* control animals were incubated at 25°C for the first 24 h of postembryonic development to eliminate germ cells, before being shifted to 20°C for the remainder of the experiment.

daf-2(e1370) worms and corresponding *daf-2(+)* control worms were continuously cultured at 20°C. To prevent progeny production in fertile strains, all worms were cultured on plates supplemented with 20 μ M 5-fluoro-2'-deoxyuridine (FUDR) from the L4 stage onwards.

Growing Worms for RNA Extraction

To obtain synchronized populations, gravid adults were treated with hypochlorite and the eggs were allowed to hatch in M9 buffer overnight. Depending on the experiment, approximately 1,200–1,800 L1 larvae per strain were plated on 10-cm NG agar plates seeded with concentrated *E. coli* OP50 and cultured at the required temperatures. At the L4 stage, 1,200–1,500 worms per strain were transferred to 6 *E. coli* OP50-seeded 6-cm NG agar plates supplemented with 20 μ M FUDR to inhibit germ cell proliferation and progeny production. The worms from 2 of these plates were harvested 24 h later by washing them off with M9. After additional washing with M9 and RNase-free water, the worms were suspended in 1 mL Tri Reagent® (Sigma), snap-frozen in liquid nitrogen, and stored at –80°C until RNA extraction. The 4 remaining plates were cultured at 20°C and 2 plates each were harvested analogously on day 5 and day 10 of adulthood.

Table 1. Function of B4GALTs in *C. elegans* and humans

Enzyme	Biosynthetic process					Substrate specificity (sugar component)	
	Glycoproteins		Glyco-lipids	Proteo-glycans	Lactose	Donor	Acceptor
	N-linked	O-linked					
BRE-4 B4GALT1	x [25] x [37–50]	x [25] x [43, 47, 49]	x [23] x [47, 51] 0 [41]	x [52, 53]	x [39, 45, 46, 51, 54]	GalNAc Gal	GlcNAc GlcNAc, Glc
B4GALT2	x [42, 43, 48–50, 55, 56]	x [43, 49]			x [51]	Gal	GlcNAc, Glc
B4GALT3	x [42, 43, 48–50]	x [43, 49]	x [51]		0 [51]	Gal	GlcNAc
B4GALT4	x [43, 49, 50] 0 [42, 48]	x [43, 49]	x [48]		0 [48]	Gal	GlcNAc
W02B12.11 B4GALT5	x [43, 50] 0 [47, 49, 57]	x [47]	(x) x [57, 58]		0 [47, 59]	(GalNAc) Gal	(GlcNAc) GlcNAc, Glc
B4GALT6	x [41, 50] 0 [60]		x [58, 60] 0 [41]			Gal	GlcNAc, Glc
SQV-3 B4GALT7				x [28] x [61–63]		Gal Gal	Xyl Xyl

Enzymes are grouped based on homology (see Fig. 1a). The reference numbers of studies providing relevant in vitro or in vivo evidence are supplied. “x” indicates activity and “0” indicates low/no activity in a particular process. Bold type indicates the major enzyme implicated in a particular process; underscore indicates the major enzymatic activity (as determined in comparative analyses). Parentheses indicate predictions based on our own sequence homology analyses and those in Ramakrishnan and Qasba [8]. The (UDP-activated) donor sugar and the (conjugated) acceptor sugar are listed. Note that Glc serves as acceptor in its free form (lactose synthase), or as component of glucosylceramide (lactosylceramide synthases B4GALT1/5/6 [51, 57, 58, 60]).

RNA Extraction

RNA was extracted using Tri Reagent® (Sigma) and cleaned up with the Monarch® Total RNA Miniprep kit (New England Biolabs) according to the manufacturers' instructions.

qPCR

1 µg total RNA was reverse-transcribed using LunaScript® RT SuperMix (New England Biolabs). qPCR reactions were performed in triplicate in 20 µL reaction volume on an CFX Connect™ real-time PCR detection system with iTaq™ Universal SYBR® Green Supermix (both from Bio-Rad Laboratories). The thermal cycling protocol comprised 1 activation step at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 10 s and combined annealing/extension at 60 °C for 30 s. Melting curve analysis was performed from 65 to 95 °C with 0.5 °C increments at 5 s per step. Data were analyzed by the $\Delta\Delta C_t$ method and target gene expression levels were normalized to the geometric mean of *cdc-42*, *tba-1*, and *Y45F10D.4* expression [33, 34]. Primer sequences are listed in online Supplementary Table S1 (see www.karger.com/doi/10.1159/000510722 for all online suppl. material).

Statistical Analysis of qPCR Data

Means and pooled standard deviations from 4 independent experiments were calculated in Excel and pasted into Prism 5 (Graph-Pad Software) for plotting and statistical analysis. Details on the particular tests used are specified in the figure legends.

Multiple Sequence Alignment

The ClustalΩ multiple sequence alignment of the 7 human and 3 *C. elegans* B4GALT proteins (Fig. 1a) was generated using the alignment tool at www.uniprot.org. The degree of identity and similarity between human and *C. elegans* B4GALTs (Fig. 1b) was determined by pairwise global sequence alignment using the same tool. The following sequences from the UniProt database were aligned (last accessed on 01.02.2020): B4GALT1: P15291; B4GALT2: O60909; B4GALT3: O60512; B4GALT4: O60513; B4GALT5: O43286; B4GALT6: Q9UBX8; B4GALT7: Q9UBV7; BRE-4: Q9GUM2; SQV-3: P34548; W02B12.11: G5EGS9.

Results

Bre-4 mRNA-Levels Increase and *sqv-3* Levels Decrease during Aging of *C. elegans*

Based on sequence analysis (OrthoList 2 [35]; our own reciprocal BLAST searches), human B4GALT1 is orthologous to *C. elegans* BRE-4. Other close relatives of BRE-4 include B4GALT2/3/4, while the 2 other *C. elegans* B4GALTs, W02B12.11 and SQV-3, are most closely related to human B4GALT5/6 and B4GALT7, respectively

(Fig. 1a). All human and *C. elegans* B4GALTs, except for W02B12.11, have been functionally characterized and form β -1,4-glycosidic bonds between donor and acceptor sugars (Table 1). To investigate how mRNA expression levels of the 3 B4GALT enzymes change during aging in *C. elegans*, we performed qPCR analysis on young adult worms (day 1), on worms at the end of the reproductive phase (day 5), and on aged worms before the onset of massive death in the population (day 10) ([36]; own observations). While mRNA levels of *bre-4* on day 5 and day 10 were similarly increased relative to day 1, by 43–58% on average, the expression of *sqv-3* decreased to 46–57% during the same time course (Fig. 1b). W02B12.11 mRNA levels, on the other hand, did not significantly differ from each other at the time points examined (Fig. 1b). In summary, the observed increase in *bre-4* mRNA levels parallels the increase of plasmatic B4GALT activity during aging in humans.

Bre-4 and sqv-3 mRNA Levels Change during Aging of Long-Lived C. elegans Mutants

To determine whether, and eventually how, mRNA levels of the 3 B4GALTs changes during the aging of long-lived *C. elegans* mutants, we examined the expression of *bre-4*, *sqv-3*, and W02B12.11 by qPCR in *daf-2(-)* (insulin/IGF1-receptor-defective) and *glp-1(-)* (germ-line-deficient) worms at the same chronological age as wild-type worms. Similar to wild-type worms (Fig. 1b), *daf-2(-)* animals displayed 69–88% elevated *bre-4* mRNA levels on day 5 and day 10, respectively (Fig. 2a). Analysis of wild-type worms grown under a different temperature regimen (*glp-1(+)*; Fig. 2b) also revealed a trend towards elevated *bre-4* mRNA expression on day 5, and detected a clear (64 %) increase on day 10, thus confirming our previous result (Fig. 1b, 2b). In contrast to wild-type and *daf-2(-)* worms (Fig. 1b, 2a, 2b), *glp-1(-)* worms on day 10 did not display an increase, but rather a trend towards decreased *bre-4* mRNA expression relative to day 1 (Fig. 2c). On the other hand, *sqv-3* expression was decreased on both day 5 and day 10 in all genetic backgrounds (to 47–58%, Fig. 2a; to 45–69%, Fig. 2b; to 76–67%, Fig. 2c). Finally, W02B12.11 mRNA expression appeared constant in the genetic backgrounds analyzed, and the reduction in *daf-2(-)* worms at the later time points did not reach statistical significance (Fig. 2). In summary, our data are consistent with the hypothesis that *bre-4* and *sqv-3*, due to age-related changes in mRNA levels, contribute to lifespan regulation, not just in wild-type, but also in long-lived *daf-2(-)* and *glp-1(-)* *C. elegans*. On the other hand, our observations do not exclude

the possibility that W02B12.11 also modulates lifespan, although by means other than changes in mRNA expression.

B4GALT mRNA Levels Are Similar between Wild-Type and daf-2(-) C. elegans

Apart from time-dependent changes, different mRNA expression levels between wild-type and long-lived *C. elegans* mutants could also hint towards a gene of interest functioning in lifespan regulation. Currently available high-throughput studies do not, or not consistently, provide evidence for altered B4GALT mRNA expression in *glp-1(-)* or *daf-2(-)* *C. elegans* relative to wild-type worms, and generally cover only early adulthood (online suppl. Table S2). Similarly, high-throughput studies on multiple lifespan-regulatory transcription factors do not, or not consistently, provide evidence for one of the B4GALTs being a transcriptional target of any of these factors in L4 or young adults, even though CHIP seq data indicates that some of these factors occupy regulatory regions of the B4GALT genes (online suppl. Tables S2, S3). To clarify potential differences in B4GALT levels between wild-type and long-lived mutants at multiple ages, we compared B4GALT mRNA levels between *daf-2(-)* or *glp-1(-)* and their corresponding wild-type control animals cultured under the same conditions. At all time points, mRNA levels of *bre-4*, *sqv-3*, and W02B12.11 did not statistically significantly differ between *daf-2(+)* and *daf-2(-)* worms (Fig. 3). On the other hand, when comparing mRNA levels between *glp-1(+)* and *glp-1(-)* worms, expression appeared reduced for W02B12.11 at all time points (to 56–68%), for *sqv-3* on days 1 and 10 (51–50%), and for *bre-4* only on day 10 (58%). Nevertheless, direct comparisons of transcript levels between *glp-1(+)* and *glp-1(-)* worms is difficult due to the absence of germ cells in the latter [64].

Discussion

In this study, we investigated the possibility that *C. elegans*, a preferred invertebrate model system for experimental research into aging [65], can also be used to study potentially conserved roles of the conserved B4GALT enzyme family during aging. Our interest in this question was sparked by a recent report that suggested B4GALT activity in plasma as a novel biomarker for human aging [6]. Our gene expression analyses presented here indicate an age-related increase in mRNA levels for *bre-4*, the *C. elegans* ortholog of B4GALT1, the major candidate for

Fig. 2. mRNA levels of *bre-4* and *sqv-3* change during aging of long-lived *C. elegans* mutants. mRNA levels of the 3 B4GALTs were measured by qPCR and normalized to housekeeping genes in *daf-2(-)* (a), *glp-1(+)*/wild-type (b), and *glp-1(-)* (c) worms on day 1, day 5, and day 10 of adulthood (see Fig. 1b for *daf-2(+)*/wild-type animals analyzed in parallel with *daf-2(-)* worms). Data rep-

resent the mean expression relative to day 1 $\pm$ SD from 4 biological replicates. Statistical significance was determined by two-way ANOVA with Bonferroni post hoc tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Please note that the legend for panel a also applies to panels b and c.

Fig. 3. mRNA levels of B4GALTs do not differ between *daf-2*(+) and *daf-2*(-) *C. elegans*. mRNA-levels of the 3 B4GALTs were measured by qPCR and normalized to housekeeping genes on day 1 (a), day 5 (b), and day 10 (c) of adulthood in the genotypes indicated. Data represent the mean expression relative to wild-type,

i.e., *daf-2*(+) or *glp-1*(+) worms, $\pm$ SD from 4 biological replicates. Statistical significance was determined by two-way ANOVA with Bonferroni post hoc tests (** $p < 0.01$, *** $p < 0.001$). Please note that the legend for panel a also applies to panels b and c.

mediating the reported age-related B4GALT activity increase in humans [6]. These increasing levels in *C. elegans* *bre-4* mRNA and human B4GALT1 activity during aging parallel each other and support the concept that *C. elegans* can serve as a suitable model for investigating B4GALT1/BRE-4 functions during aging. To our knowledge, our study is the first targeted analysis of B4GALT expression during aging in *C. elegans*, and therefore validates an earlier high-throughput analysis that already

provided evidence for an age-dependent increase of *bre-4* levels in wild-type animals [66]. On the other hand, that particular study did not report on *W02B12.11* and suggested *sqv-3* levels to be slightly increased, while we observed a robust decrease in *sqv-3* mRNA during aging in 3 genetic backgrounds. The reason for this discrepancy remains to be determined.

Beyond sequence homology, *C. elegans* and human B4GALTs share overlapping tissue expression patterns.

BRE-4, SQV-3, and W02B12.11 are, apparently, broadly expressed across tissues and throughout all stages of the life cycle [67, 68]. Similarly, B4GALT1–7 mRNAs and proteins are detected in a broad variety of human tissues and cell types, including tissues already present in *C. elegans* [69]. Examples include skeletal muscle, the reproductive system, and the nervous system as well as gut, liver, and adipose tissue, which all correspond to 1 multifunctional *C. elegans* tissue, the intestine [70]. In addition, B4GALT1/BRE-4 appear similar to each other based on their age-related changes in activity/expression ([6]; this study), even though additional studies are required to investigate the mechanistic cause in humans, the functional consequence in *C. elegans*, and the suitability of *bre-4* as a biomarker also for *C. elegans* aging. On the other hand, B4GALT1 and BRE-4 differ in their precise enzymatic activities [8, 25]. A catalytically important amino acid in B4GALTs changed during evolution (from Ile/Leu to Phe/Tyr) and shifted donor sugar preference from UDP-GalNAc in invertebrate enzymes, including BRE-4, to UDP-Gal in vertebrate enzymes such as human B4GALT1–6 [8] (also Fig. 1a; Table 1). Clearly, during aging and beyond, it will be interesting to determine BRE-4/B4GALT1 substrates, and whether differences in the precise nature of BRE-4/B4GALT1-dependent glycoconjugates are functionally important. Yet, potentially shared cellular/organismal functions of B4GALT1/BRE-4 may as well rely on their nonenzymatic activities, such as protein-protein interactions. In line with this possibility, B4GALT-dependent oligosaccharide structures on immunoglobulins were not observed to be increased during human aging, despite the increase in B4GALT activity [6]. Furthermore, BRE-4 was demonstrated to share binding properties with human B4GALTs, at least in vitro, as it also binds to human α -lactalbumin, a member of the lysozyme protein family already present in *C. elegans* [25, 71].

We observed an age-related increase in *bre-4* mRNA levels in wild-type *C. elegans*, but also in long-lived *daf-2(-)* worms. Remarkably, in another type of long-lived *C. elegans* mutant, *glp-1(-)*, *bre-4* expression in older (day 10) worms is not elevated relative to young (day 1) worms. Therefore, *bre-4* expression is responsive to at least 1 lifespan-regulatory pathway, namely that triggered by germline ablation. This observation supports the hypothesis that altered *bre-4* expression is functionally important for lifespan regulation, at least in certain genetic backgrounds. Of note, at least 2 *bre-4* regulated processes, N-linked protein glycosylation and lipid glycosylation, already have been implicated in lifespan regulation through

genetic, functional, and proteomic analyses in *C. elegans* [72–75].

Similarly, lower levels of *sqv-3* mRNA in older wild-type *C. elegans* raise the possibility that this enzyme modulates lifespan. In a simple model, loss of *sqv-3* may contribute to aging, but long-lived *daf-2(-)* and *glp-1(-)* worms, that also display lower *sqv-3* mRNA levels at an older age, may be able to buffer this loss. Intriguingly, mutations in SQV-3's human ortholog B4GALT7 are the molecular cause of type 1 progeroid Ehlers-Danlos syndrome (spondylodysplastic EDS due to B4GALT7 deficiency) [61, 76]. SQV-3/B4GALT7 display the same enzymatic activity, including preference for the same donor and acceptor sugar, UDP-Gal and Xyl, respectively [8], and both contribute to the synthesis of proteoglycans [28, 61, 63]. Given these broad overlapping activities of SQV-3 and B4GALT7, functional studies to determine how altered *sqv-3* expression modulates adult lifespan in *C. elegans* seem extremely promising to uncover conserved mechanisms of normal aging.

In summary, the experimental data we report here point towards a remarkable conservation of the age-related B4GALT activity increase in species as diverse as *C. elegans* and humans. Our results are consistent with the concept that *C. elegans* provides a suitable model system to study potentially conserved functions of B4GALTs, in particular BRE-4/B4GALT1 and SQV-3/B4GALT7, during aging. Therefore, our observations clearly encourage further mechanistic studies on B4GALTs during *C. elegans* aging, which may also shed light on their role during normal and accelerated aging in humans.

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Statement of Ethics

Ethics approval was not required for the studies reported here on the invertebrate *Caenorhabditis elegans*.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest relating to this manuscript.

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Author Contributions

C.B. and H.I.D.M. conceived the project. E.K.M.M. and H.I.D.M. designed experiments. J.K. and H.I.D.M. performed experiments. J.K., E.K.M.M. and H.I.D.M. analyzed the data. E.K.M.M. and H.I.D.M. drafted the manuscript. All authors revised the manuscript.

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