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Article

Identification of a Glucuronyltransferase-Related Gene, *GlcAT-S* with Putative Mucus Protection and Anti-Inflammatory Effects from Gut-Damaged *Drosophila* by DSS

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Simple Summary: The present study provides evidence that *GlcAT-S* plays a protective role in the maintenance of gut tissue integrity and the regulation of intestinal inflammation in a *Drosophila* gut-damage model. The disruption of the mucosal barrier is a key feature of inflammatory bowel disease, and understanding the mechanisms underlying this disruption and the subsequent inflammatory response is crucial for developing effective therapies. Using a combination of bioinformatics analysis and genetic manipulation, we identified *GlcAT-S* as a key gene involved in mucosal protection and demonstrated its role in DSS-induced intestinal damage. Our findings suggest that *GlcAT-S* may serve as a potential therapeutic target for inflammatory bowel disease treatment.

Abstract: The intestinal epithelium, protected by mucosal surfaces composed of mucins and other glycoproteins, functions as a selective barrier that absorbs nutrients while preventing the translocation of harmful substances. To understand the mechanisms between mucosal disruption and tissue inflammation, we orally administered mucus-disrupting agent, dextran sodium sulfate to *Drosophila melanogaster* and screened 63 differentially expressed genes (DEGs). Through database search using bioinformatics tools (CHEA3 and WebGestalt), we identified ELK1 as a potential key transcription factor for the selected DEGs, and among the 63 DEGs, ELK1-related genes, B3GAT3, FIBP, and TENT2 (*GlcAT-S*, *Fibp*, and *Wisp* in *Drosophila*), were selected as relevant genes responding to mucus disruption. We confirmed that enterocyte (EC)-specific *GlcAT-S* knockdown by RNAi significantly reduced gut length and increased intestinal stem cell proliferation in *Drosophila*. Additionally, in EC-specific *GlcAT-S* knockdown flies, it was observed that the mucus production-related gene, *Muc68D* and *Mur29B*, were specifically reduced, whereas the inflammatory cytokines *egr* and *upd3*, were overexpressed. This study provides evidence that *GlcAT-S* is involved in the regulating intestinal inflammation in *Drosophila* and plays a protective role against mucus disruption. Our findings suggest that *GlcAT-S* may be a potential therapeutic target for the treatment of intestinal inflammatory diseases such as IBD.

Keywords: *Drosophila* gut-damage model; Bioinformatics tools; Inflammation; *GlcAT-S*; Mucin

1. Introduction

IBD is a chronic, multifactorial condition that is characterized by inflammation of the tissue and increased permeability [1]. Disruption of the mucosal barrier is a hallmark of IBD and is closely associated with disease progression and pathogenesis [2]. Intestinal mucus layer plays a crucial role

in maintaining gut homeostasis and protecting against tissue damage [3]. In conditions such as ulcerative colitis, alterations in mucus composition, thickness, or continuity can lead to increased bacterial penetration and subsequent inflammation [3]. However, IBD is caused by various factors such as organ failure, chemical exposure, microbiota disruption, stress, unhealthy diet, and surgery [4]. Therefore, identifying key genes involved in gut tissue inflammation following mucosal disruption could be essential in understanding the mechanism of the complex disease.

Drosophila melanogaster model is a powerful model organism for studying a wide range of human disease, due to its high similarity of human disease-related genes [5]. Especially, *Drosophila* gut has a similar tissue structure, cellular components, and gene response with mammals, therefore it is used as gut disease model [6]. The *Drosophila* midgut shares molecular and cellular homology with mammalian intestines, making it a robust model for studying gut regeneration, barrier function, and inflammation [6]. Equivalent to the mammalian gut mucus layer, a peritrophic matrix (PM), acting as a physical defense, is present in the *Drosophila* intestine [7]. PM is a highly organized layer that is composed of glycosaminoglycan (GAG) and chitin [8], that are synthesized by multiple genes and enzymes such as glucuronyltransferases. Oral administration of dextran sulfate sodium (DSS) is widely used to induce gut inflammation by disrupting the mucosal barrier, this model provides insights into the mechanisms of intestinal inflammation and potential therapeutic targets for IBD treatment [9]. The DSS is the most widely used to achieve IBD murine model [10] which also applicable to *Drosophila* to induce IBD model [11]. *Drosophila* intestinal epithelium possesses a complex protective system, featuring both a semi-permeable PM of chitin and proteins, and a thin mucus layer, detectable by periodic acid-Schiff (PAS) staining [12][13]. Thus, oral-administrated DSS *Drosophila* is reliable IBD model. Moreover, *Drosophila* is amenable to genetic manipulation, allowing for the generation of transgenic flies that express specific genes or RNAi constructs [14]. This feature makes *Drosophila* an ideal model for studying the role of specific genes, in the pathogenesis of IBD. However, the exact mechanism of GAGs in mucus disrupted model remain incompletely understood.

RNA-sequencing provides very large amounts of gene expression data, thus, the characterization of differentially expressed genes (DEGs) through bioinformatics tools are a widespread method due to its efficiency [15]. Bioinformatics tools are powerful methods for analyzing large amounts of genetic data, and screening core genes, biomarkers, proteins, metabolites, and microRNAs [16]. Recently, bioinformatics tools have played a crucial role in discovering key genes in the certain multifactorial diseases such as cancer, diabetes, epilepsy, and inflammatory bowel disease (IBD) [17]. DEG analysis is a molecular biology method used to compare gene expression levels between two or more sample groups, such as healthy versus damaged tissues under different treatment conditions [18]. These databases facilitate the annotation, classification, pathway mapping, and gene function, aiding in the screening of core genes from large genetic datasets.

In this study, we used bioinformatics tools to screen DEGs from sequencing data of a DSS-induced *Drosophila* gut-damage model and investigated the role of *GlcAT-S* in intestinal inflammation in this model. We generated enterocyte (EC)-specific *GlcAT-S* mutant flies and assessed their susceptibility to DSS-induced intestinal inflammation. Our results demonstrate that *GlcAT-S* mutant flies are more susceptible to DSS-induced intestinal inflammation, suggesting that *GlcAT-S* plays a protective role against gut tissue inflammation. This study provides the first evidence that *GlcAT-S* is involved in the regulation of intestinal inflammation in *Drosophila*. Our findings suggest that *GlcAT-S* may be a potential therapeutic target for the treatment of IBD.

2. Materials and Methods

2.1. Experimental *Drosophila* and Feeding Assay

Drosophila melanogaster was reared on a standard cornmeal-yeast-sucrose diet at 18 °C until the initiation of feeding experiments. Adult flies were collected at a 1:1 male-to-female ratio using light CO₂ anesthesia and maintained under controlled conditions (25 °C, 65% relative humidity, 12 h light/dark cycle) until use in experiments. For feeding assays, groups of 50 flies (25 males and 25

females) were housed in vials containing chromatography paper (2.5 cm × 3.75 cm) soaked with 500 µL of the specified test solutions. The control group received 5% sucrose, while experimental groups were exposed to different treatments: 5% sucrose with 7% dextran sulfate sodium (DSS, MW 40 kDa; MP Biomedicals, Solon, OH, USA) to induce gut damage. Feeding experiments were conducted at 29 °C. Vials and media-soaked papers were replaced daily, and mortality was recorded throughout the study.

2.2. cDNA Synthesis and Quantitative PCR Analysis

Drosophila midguts were dissected and homogenized, followed by total RNA extraction using the TRIzol reagent (Ambion, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted RNA was further purified using lithium chloride precipitation solution (Invitrogen, Carlsbad, CA, USA) to enhance purity and integrity. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the QuantiNova Reverse Transcription Kit (QIAGEN GmbH, Hilden, Germany), following the manufacturer's instructions. Quantitative polymerase chain reaction (qPCR) was conducted using the QuantiNova SYBR Green PCR Kit (QIAGEN GmbH, Hilden, Germany) on CFX96 thermocycler (Bio-Rad Laboratories, Hercules, CA, USA).

2.3. EC-Specific Knockdown of Selected Genes Using RNA-Interference

We specifically knocked down gene expression in ECs using the Myo31DF-GAL4 driver line [19], in conjunction with a temperature-sensitive tubGal80^{ts} driver line (#67067, Bloomington *Drosophila* stock center (BDSC), Indiana, USA) [20]. Temperature-sensitive tubGal80^{ts} suppresses GAL4 at 18 °C by expressing Gal80, while tubGal80^{ts} is inactivated at 29 °C, thus inducing GAL4 expression. Three RNAi lines were obtained from the BDSC: GlcAT-S (#67781), Fibp (#63665), and Wisp (#43141). Male UAS-RNAi transgenic flies were crossed with virgin driver line females at a 1:3 ratio. The resulting F1 progeny and all crosses were reared at 18 °C until adulthood, and 3- to 5-day-old adults were subsequently shifted to 29 °C for 2 days to induce RNAi expression by inhibiting Gal80.

2.4. *Drosophila* gut Immunostaining and Imaging

Drosophila midguts were dissected in ice-cold 4% paraformaldehyde (PFA) and fixed at room temperature for either 20 min or 60 min, depending on the experimental conditions. Following fixation, samples were washed three times with phosphate-buffered saline (PBS) (10 min each), followed by two washes in PBS containing 0.1% Triton X-100 (PBST) (30 min each). To reduce non-specific binding, samples were blocked for 90 min in a blocking buffer (PBST supplemented with 3% bovine serum albumin). For phospho-histone 3 (PH3) staining, samples were incubated overnight at 4 °C with primary antibody (rabbit anti-PH3, 1:600, Cell Signaling Technology, Beverly, MA, USA) diluted in blocking buffer. The following day, samples were washed four times in PBSTA (PBST containing 0.5% bovine serum albumin) (20 min each) and incubated with secondary antibody (goat anti-rabbit IgG Alexa Fluor 568, 1:2000, Invitrogen, Rockford, IL, USA) for 180 min at room temperature. After secondary antibody incubation, samples were washed three times in PBST (20 min each), briefly rinsed in PBS three times, and mounted on slides using VECTASHIELD PLUS mounting medium containing DAPI (Vector Laboratory, Burlingame, CA, USA). For imaging, samples were visualized using an Olympus BX51 fluorescence microscope (Olympus Optical Co., Tokyo, Japan) equipped with a JNOPTIC BKONV KON3.1 CMOS camera (JNOPTIC Co., Seoul, Korea). Female *Drosophila* gut lengths were measured using JNO-ARM software (JNOPTIC Co., Seoul, Korea) by tracing segmented curves along the center of each midgut (from end of proventriculus to midgut-hindgut junction). For each group, five guts were initially measured, then the longest and shortest guts were excluded, resulting in n=3. Intestinal stem cells (ISCs) were quantified by counting PH3-positive cells in the entire midgut. Imaging parameters were standardized across all samples, and cell counts were averaged from five different regions per experimental group.

2.5. Statistical analysis

All statistical analyses were performed using Student's *t*-test in GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA), with significance defined as $p < 0.05$.

3. Results

3.1. Identification of key DEGs from DSS- induced *Drosophila* IBD model through multiple bioinformatics tools

In IBD, disruption of the mucus layer is a common feature, leading to increased gut permeability, heightened immune responses, and chronic inflammation [4]. We previously identified DEGs in *Drosophila* following oral administration of the mucus-disrupting agent DSS [21]. First, we converted the *Drosophila* DEGs to their human orthologs by Isobase [22], resulting in 41 upregulated and 22 downregulated DEGs (Figure 1A). To account for incomplete network databases in bioinformatics tools, we used two different tools, CHEA3 (ChIP-X Enrichment Analysis) [23] and WebGestalt (WEB-based Gene Set Analysis Toolkit) [24], and screened for overlapping genes via transcription factor (TF) enrichment analysis to improve the accuracy of essential gene identification. Using these bioinformatics tools, ELK1 was the only overlapping TF identified (Figure 1B). Furthermore, from the selected DEGs (Figure 1A), we identified 3 genes (B3GAT3, Beta-1,3-Glucuronyltransferase 3; FIBP, FGF1 Intracellular Binding Protein; and TENT2, Terminal Nucleotidyltransferase 2) that are related to ELK1 as a TF (Figure 1C). B3GAT3 and their family (B3GAT2) are gene that encodes an enzyme for biosynthesis of GAGs that catalyzes glycosaminoglycan-protein linkage [25]. FIBP encodes the acidic fibroblast growth factor intracellular-binding protein, which plays several important roles such as mitogenesis, embryonic development and colorectal cancer chemoresistance [26]. TENT2 is a cytoplasmic poly(A) RNA polymerase that plays crucial roles in RNA modification and regulation [27]. Since B3GAT families are involved in GAG biosynthesis, it may be related to the PM and mucus. However, such associations have not yet been reported for the other genes.

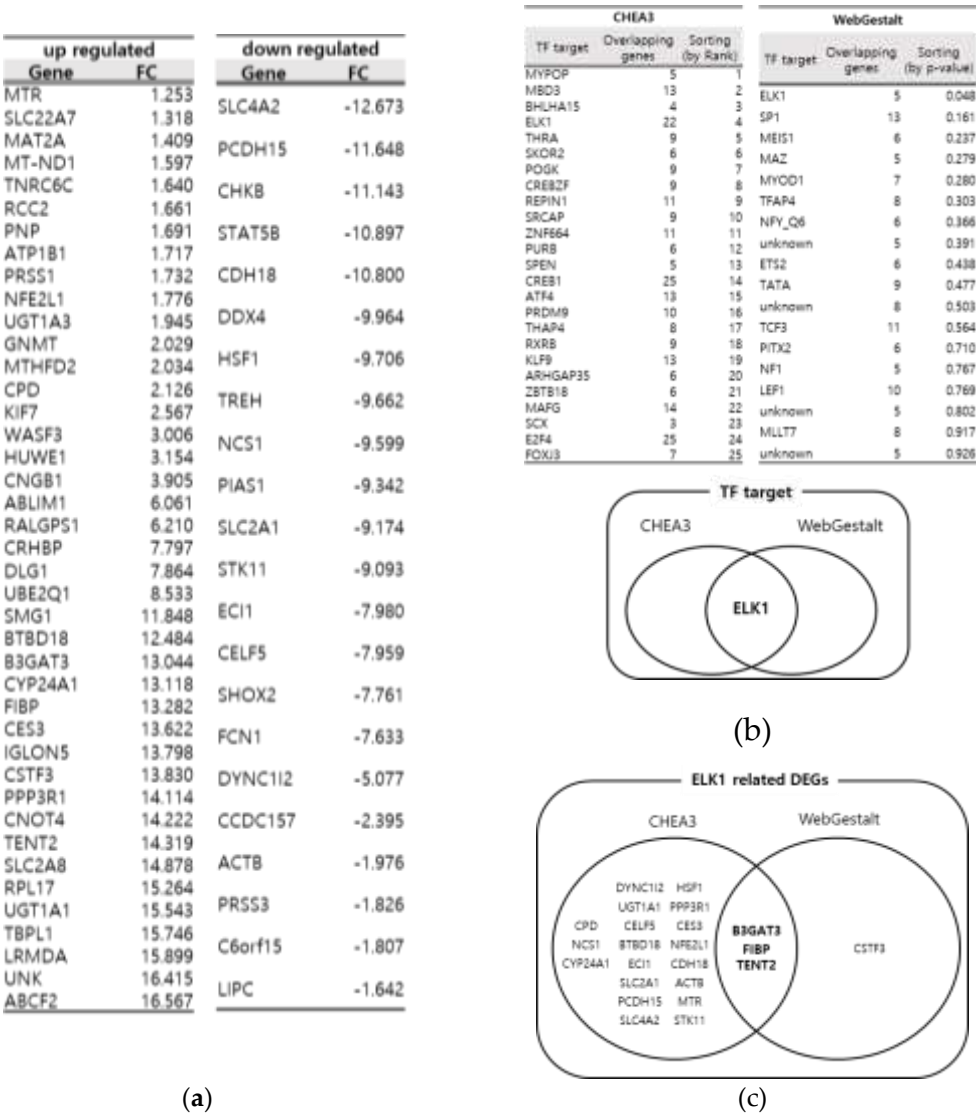


Figure 1. Essential gene screening of *Drosophila* mucus disruption model by bioinformatics tools. (a) Differentially expressed genes (DEGs) were identified from sequencing data of DSS-administered *Drosophila*, with ortholog conversion via Isobase. 41 genes were upregulated and 22 genes were downregulated. DEGs were selected by certain significance level (Log fold change ≥ 11 , false detection rate < 0.01). (b) Total DEGs were then screened using two different transcription factor target databases, CHEA3 and WebGestalt, revealing ELK1 as a common transcription factor target. (c) Further analysis of ELK1-related genes within our DEGs identified 22 genes from the CHEA3 database and 4 genes from the WebGestalt database. Notably, B3GAT3, FIBP, and TENT2 were identified as overlapping genes (*GlcAT-S*, *Fibp*, and *Wisp* in *Drosophila*).

3.2. EC-Specific Knockdown of Screened DEGs

To determine whether the 3 screened genes B3GAT3, FIBP, and TENT2 (*GlcAT-S*, *Fibp*, and *Wisp* in *Drosophila*) play a role in gut tissue during DSS-mediated injury. Because ECs are the major cell type in the gut tissue and are responsible for nutrient absorption, secretion, defense, and barrier function, we generated EC-specific RNAi lines to knock down the expression of these 3 genes in ECs and analyzed their gut tissue [28], we constructed EC-specific RNAi lines for the knockdown of selected 3 genes in EC and analyzed their gut tissue. We confirmed the transcription expression level of 3 DEGs in the gut tissue of each EC-specific RNAi line by qRT-PCR, respectively (Figure 2A). Gut length is reduced following damage and is therefore an important indicator of tissue health [29]. Among the three RNAi knockdown lines, only *GlcAT-S* RNAi flies exhibited a significantly shortened

gut length (4345.1 μm) compared to the control group (6267.2 μm). These results suggest that among the three screened DEGs, *GlcAT-S* is more important in gut tissue maintenance.

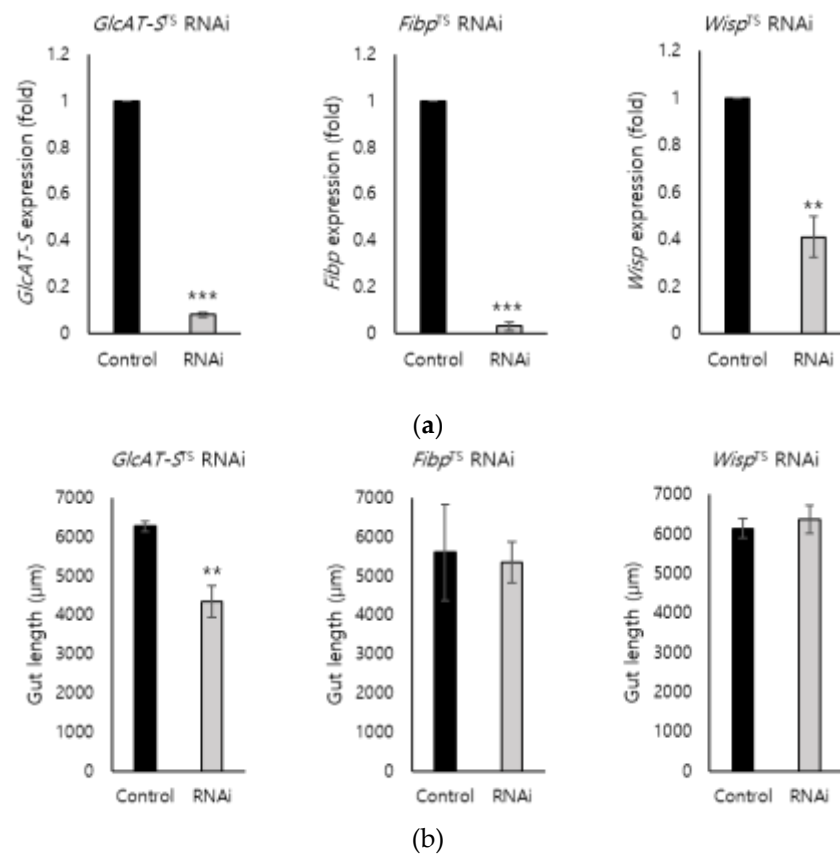


Figure 2. Enterocyte (EC)-specific knockdown of three screened DEGs. To investigate the roles of three genes (*GlcAT-S*, *Fibp*, and *Wisp*) in ECs, we performed EC-specific knockdown using RNA interference (RNAi) with a temperature sensitive Myo1A driver line. (a) Successful knockdown of each gene in gut tissue was confirmed using qPCR. (b) Measurement of gut length in the knockdown lines revealed a significant shortening of the gut specifically in flies with EC-specific *GlcAT-S* knockdown. Error bars represent SD (n = 3, *p < 0.05, **p < 0.01, ***p < 0.001, t-test).

3.3. Confirmation of the Relationship Between *GlcAT-S* and Gut Tissue damage Through EC-Specific Knockdown

Increased numbers of *Drosophila* intestinal stem cells (ISCs) are a hallmark of epithelial cell (EC) damage, reflecting a tissue repair mechanism [30]. PH3 is a widely used marker for mitotic cells, in *Drosophila* midgut, only ISCs proliferate and exhibit PH3 mitotic activity [31]. To confirm the importance of *GlcAT-S* in protecting against gut tissue damage, we quantified PH3-positive cells in the midgut of flies following EC-specific *GlcAT-S* knockdown. The survival rate of EC-specific *GlcAT-S* RNAi flies was significantly reduced to 21.3% after 10 days, compared to 94.7% in control flies (Figure 3A). Furthermore, the number of PH3+ cells was dramatically increased, with 35.3 PH3+ cells per *GlcAT-S* RNAi fly midgut compared to 2.7 PH3+ cells in control flies. Together, these data indicate that reduced EC-specific expression of *GlcAT-S* is associated with gut damage and leads to a decreased survival rate.

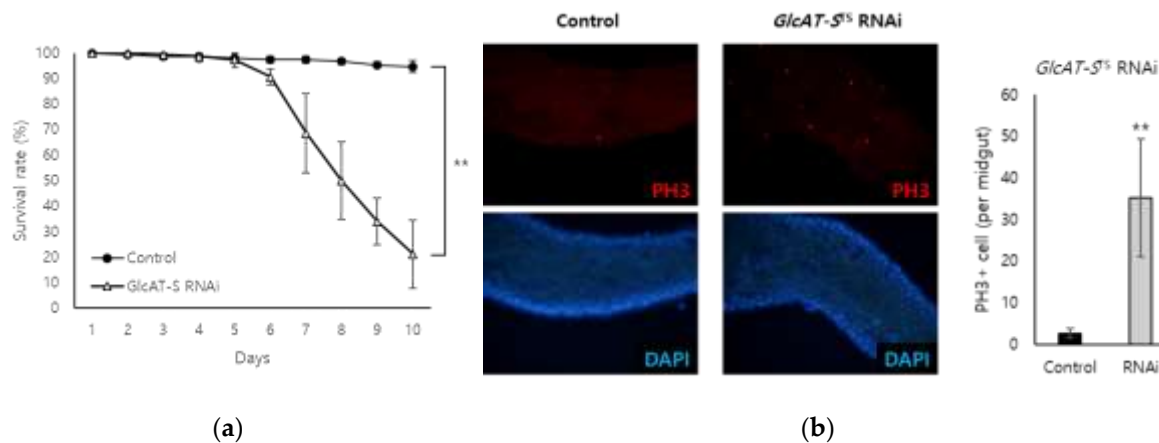


Figure 3. *Drosophila* EC-specific knockdown of *GlcAT-S* caused gut damage. To evaluate the roles of *GlcAT-S* in EC, EC-specific RNAi was induced by temperature sensitive Myo1A driver line. (a) After 10 days, survival rate of *Drosophila* was significantly reduced to 21.3% following *GlcAT-S* knockdown. (b) Intestinal stem cell (ISC) proliferation was labeled by phosphor-histone H3 (PH3) mitosis marker. ISC proliferation increased significantly to 35.3 cells (per gut) after EC-specific *GlcAT-S* knockdown, indicating gut damage and EC-derived cytokine release. Error bars represent SD (n=3, **p < 0.01, t-test compared with control group).

3.4. Analysis of the Relationship Among *GlcAT-S*, Mucus Loss, and Inflammation in the *Drosophila* Gut-Damage Model

Inflammation is a complex biological response to harmful stimuli such as pathogens or damaged cells [32] and is a key component of the immune system's defense mechanism, especially in gut tissue [33]. The mucus layer in the gut is a critical barrier that protects the underlying epithelial cells from damage [34]. Mucus loss is a common feature of IBD, and it can contribute to inflammation by allowing bacteria and other harmful substances to contact the epithelial cells [35]. Oral-administration of DSS disrupt the mucus loss in the gut tissue [36]. We hypothesized that altered *GlcAT-S* expression in DSS-induced *Drosophila* would be closely related to inflammation in ECs and to mucus formation covering the intestinal mucosa, as *GlcAT-S* is involved in the synthesis of various GAGs [37]. Through analysis of the Comparative Toxicogenomics Database (CTD), we investigated that human ortholog genes, B3GAT1, B3GAT2, and B3GAT3 of *GlcAT-S* are associated with human inflammation (Figure 4A). Therefore, in this study, we analyzed whether *GlcAT-S* is related to inflammation not only human but also in the fly gut (Figure 4B). EC-specific knockdown of *GlcAT-S* increased cytokine expression in fly gut tissue. The *Drosophila* TNF ortholog, *eiger* (*egr*), and the IL-6 ortholog, *unpaired 3* (*upd3*), are important signaling molecules that act as cytokines. In *GlcAT-S* RNAi flies without DSS treatment, *egr* was upregulated by 2.47-fold and *upd3* was upregulated by 2.05-fold compared to the control group. In *GlcAT-S* RNAi flies administered orally with DSS, *egr* and *upd3* were induced to express up to 7.29- and 4.85-fold, respectively, which were higher than those in the *GlcAT-S* knockdown group without DSS. These results suggest that increased inflammation in gut tissue by decreased expression of *GlcAT-S* in EC is further exacerbated by DSS. Intestinal epithelial cells constantly interact with their cellular environment, including components such as mucins and GAGs [38]. Both play crucial roles in maintaining the intestinal barrier function and mucosal immunity, alteration of mucin [10] and GAG expression in intestinal cell environment is associated with intestinal inflammation [39]. In *Drosophila*, Mucin 68D (*Muc68D*) and Mucin related 29B (*Mur29B*) are the most specific mucin genes and are abundantly expressed in the fly intestine [40]. Given that *GlcAT-S* was identified from DSS-treated, mucus-disrupted flies, we examined *Muc68D* and *Mur29B* gene expression in the gut tissue. EC-specific knockdown of *GlcAT-S* specifically downregulated *Muc68D* to 0.03-fold and *Mur29B* to 0.44-fold, compared to the DSS-treated group (Figure 4B). These results indicate that *GlcAT-S* supports the mucus layer, preventing pathogen and harmful substance

contact with epithelial cells, and that loss of *GlcAT-S* disrupts this protective barrier, leading to increased cytokine production and reduced mucin gene expression. In short, these data indicate that *GlcAT-S* plays a protective role in *Drosophila* gut tissue.

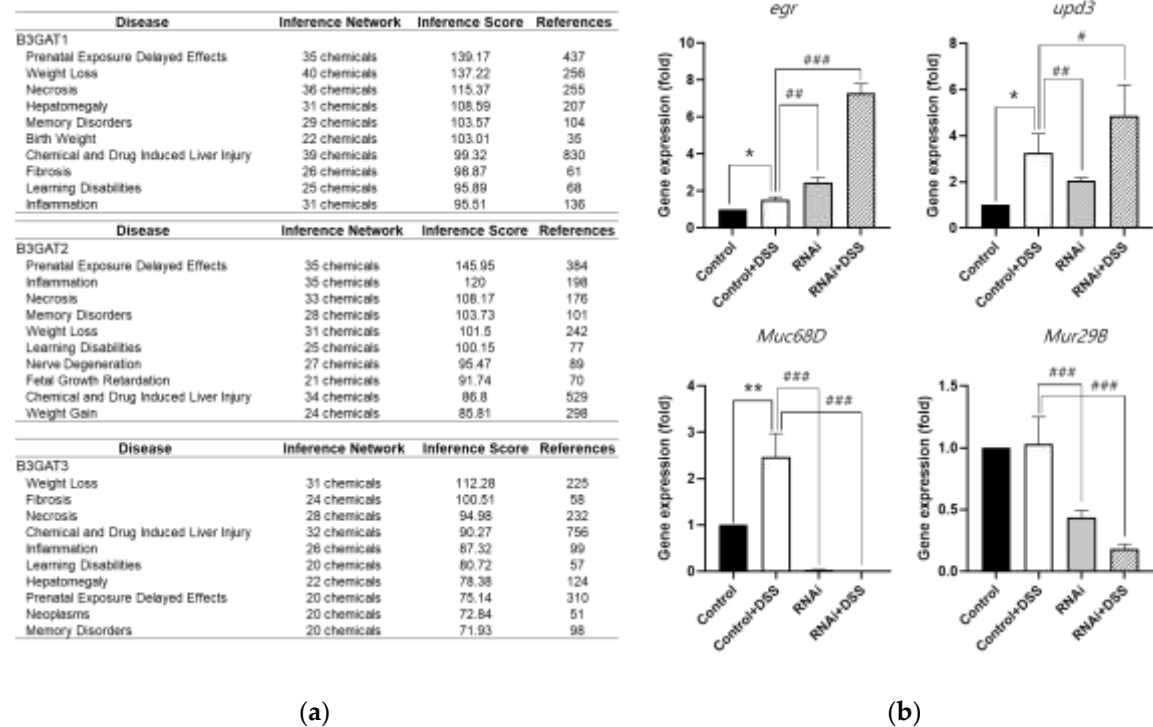


Figure 4. The inflammatory cytokine expression of EC-specific *GlcAT-S* RNAi *Drosophila* gut tissue. (a) *GlcAT-S* orthologs and related family members B3GAT1, B3GAT2, and B3GAT3 are strongly associated with human inflammation. The gene-disease relationship was confirmed from Comparative Toxicogenomics Database (CTD) bioinformatics database. (b) The expression levels of two *Drosophila* cytokines, *eiger* (*egr*) and unpaired3 (*upd3*) as well as mucus related genes *Muc68D* and *Mur29B*, were measured in gut tissue. Error bars represent SD (n=3, **p < 0.01, t-test compared with control group).

4. Discussion

To identify potential regulators of intestinal inflammation, we employed a multi-faceted approach integrating data from a DSS-induced *Drosophila* IBD model and bioinformatics tools (CHEA3 and WebGestalt). This *in silico* analysis enabled the identification of ELK1 as a potential key transcription factor influencing gut inflammation (Figure 1). Subsequently, we narrowed our focus to ELK1-related genes: B3GAT3, FIBP, and TENT2 (corresponding to *GlcAT-S*, *Fibp*, and *Wisp* in *Drosophila*). An RNAi-based screening approach in *Drosophila* then revealed *GlcAT-S* as a potential regulator of intestinal inflammation (Figure 2, 3, 4). The observed reduction in gut length in *GlcAT-S* knockdown flies compared to controls suggests a critical role for *GlcAT-S* in maintaining gut tissue integrity (Figure 2). Gut shortening is a recognized hallmark of tissue damage and impaired epithelial renewal [29]. Our finding aligns with this, underscoring the importance of *GlcAT-S* in sustaining gut homeostasis under inflammatory conditions. The *GlcAT-S* gene encodes glucuronyltransferase S, an enzyme involved in the biosynthesis of glycosaminoglycans (GAGs) such as heparan sulfate and chondroitin sulfate [41]. GAGs are essential components of the extracellular matrix and play a key role in mucosal protection [42]. They contribute to the structure and function of proteoglycans, including those that interact with mucins to maintain the viscosity and stability of the mucus layer, which acts as a barrier protecting the underlying epithelial cells from damage [43, 44]. Furthermore, previous studies have reported the upregulation of *GlcAT-S* by inflammatory cytokines, suggesting its potential involvement in the inflammatory response [45]. Elevated PH3 staining in *GlcAT-S* knockdown flies (Figure 3) indicates an increase in ISCs proliferation [30], further

supporting the idea that *GlcAT-S* is essential for preventing gut damage. ISCs are responsible for regenerating damaged epithelial cells [46]; therefore, their increased proliferation suggests a response to tissue damage caused by *GlcAT-S* deficiency. The reduced survival rate of *GlcAT-S* knockdown flies further underscores that this gene is important not only for gut health but also for overall survival. These findings suggest that *GlcAT-S* plays a protective role in the gut, and its absence leads to increased susceptibility to tissue damage and reduced survival. The evolutionary conservation of GAG biosynthesis pathways suggests that *GlcAT-S* orthologs in humans, such as B3GAT1, B3GAT2, and B3GAT3, may play similar roles in intestinal homeostasis and inflammation [39]. Our bioinformatics analysis using the Comparative Toxicogenomics Database (CTD) supports this hypothesis (Figure 4A), as these human genes are highly associated with inflammation-related pathways. Importantly, *GlcAT-S* knockdown resulted in a significant upregulation of the inflammatory cytokines, *egr* and *upd3*, and the effect was amplified by DSS administration (Figure 4). This suggests that *GlcAT-S* deficiency not only predisposes the gut to damage but also promotes inflammatory responses in a mucus-disrupted environment. Additionally, we observed a significant downregulation of the key mucin genes *Muc68D* and *Mur29B* in EC-specific *GlcAT-S* knockdown flies (Figure 4). Given that mucins are major components of the mucus layer that protects the gut epithelium from damage [3, 34, 36], this finding suggests that *GlcAT-S* is essential for maintaining mucus layer integrity. This connects *GlcAT-S* to the pathology of IBD, where mucus disruption plays a central role in disease progression.

The identification of *GlcAT-S* as a protective factor against intestinal inflammation has implications for understanding IBD pathogenesis. Our results suggest that *GlcAT-S* plays an important role in suppressing inflammatory responses. Given its role in GAG biosynthesis, alterations in GAG composition may be a contributing factor to IBD development. Furthermore, the increased susceptibility of *GlcAT-S* knockdown flies to DSS-induced inflammation suggests that individuals with impaired *GlcAT-S* function may be at an increased risk for developing IBD or experiencing more severe disease progression. These findings could potentially be used to inform personalized medicine approaches in IBD treatment and prevention. These results suggest that the integrated strategy of *in silico* prediction followed by *in vivo* validation used in this study is effective in identifying novel regulators of gut homeostasis.

5. Conclusions

Our study highlights the protective role of *GlcAT-S* in mitigating intestinal inflammation and preserving gut tissue integrity in a *Drosophila* model of IBD. By demonstrating that *GlcAT-S* knockdown exacerbates gut damage, increases inflammatory cytokine expression, and disrupts mucus homeostasis, we provide novel insights into the molecular mechanisms underlying IBD pathogenesis. These findings indicate that further exploration of *GlcAT-S* and its human orthologs as potential therapeutic targets for the treatment of IBD and other gastrointestinal disorders is warranted. Future research should focus on elucidating the exact molecular interactions between *GlcAT-S* and inflammatory pathways and on exploring pharmacological strategies to modulate its activity in disease contexts.

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