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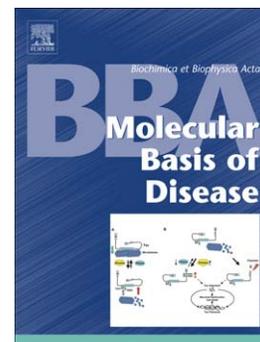
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***Caenorhabditis elegans* as a model for lysosomal storage disorders.**

Gert de Voer<sup>1</sup>, Dorien Peters, Peter E.M. Taschner<sup>#</sup>

Department of Human and Clinical Genetics, Leiden University Medical Center,  
Leiden, The Netherlands

<sup>1</sup>Present address: Genzyme Nederland, Naarden, The Netherlands

<sup>#</sup> Author responsible for correspondence: Peter E. Taschner

Department of Human Genetics

Center for Human and Clinical Genetics

Postzone S-04-P

P.O. Box 9600

2300 RC Leiden

The Netherlands

Telephone +31 (0)71-5269424

Fax +31 (0)71-5268285

e-mail: [P.Taschner@lumc.nl](mailto:P.Taschner@lumc.nl)

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**ABSTRACT**

The nematode *C. elegans* is the simplest animal model available to study human disease. In this review, the worm homologues for the 58 human genes involved in lysosomal storage disorders and for 105 human genes associated with lysosomal function have been compiled. Most human genes had at least one worm homologue. In addition, the phenotypes of 147 mutants, in which these genes have been disrupted or knocked down, have been summarized and discussed. The phenotypic spectrum of worm models of lysosomal storage disorders varies from lethality to none obvious, with a large variety of intermediate phenotypes. The genetic power of *C. elegans* provides a means to identify genes involved in specific processes with relative ease. The overview of potential lysosomal phenotypes presented here might be used as a starting point for the phenotypic characterization of newly developed knockout models or for the design of genetic screens selecting for loss or gain of suitable knockout model phenotypes. Screens for genes involved in lysosomal biogenesis and function have been performed successfully resulting in the *cup* and *glo* mutants, but screens involving subtle phenotypes are likely to be difficult.

## INTRODUCTION

### The lysosome

In 1949, Christian De Duve discovered the cellular bodies in which digestion occurs and called them lysosomes, since they contained mixtures of lytic enzymes (reviewed in [1]). These organelles were identified due to the application of different homogenization procedures. Gentle homogenization allows the lysosomes to stay intact and, therefore, facilitates retention of the enzymatic activities inside, whereas drastic homogenization causes disruption of the lysosomal membranes permitting measurements of lysosomal enzyme activity. De Duve [1] drew a parallel between the digestive tract of a multicellular organism and the “digestive tract” of a cell, both having a resistant envelope with multiple functions, e.g., to protect the rest of the organism or cell from digestion, uptake and secretion of compounds, and to maintain the degradative environment. Moreover, he reasoned that defective functioning of lysosomes could possibly lead to incomplete breakdown and subsequent accumulation of the indigestible substance, eventually causing the cell that harbors the defective lysosomes to become inoperative or even go into apoptosis. Since De Duve’s original discovery, significant progress has been made in elucidating the processes taking place in the lysosomes, although much is still unknown.

### Lysosomes and degradation and transport

Most eukaryotic cells contain lysosomes or organelles of overlapping function, which exist in a variety of shapes and sizes. These acidic degradative organelles are surrounded by a single lysosomal membrane and have a pH of about 5, which is lower than the cytoplasmic pH of approximately 7.2. They contain 50-60 hydrolytic enzymes, collectively called the acid hydrolases that usually reside in the lysosomal

lumen and have optimal activities at luminal pH. In contrast, the lysosomal membrane proteins are functional in a variety of processes such as lysosome biogenesis, maintenance of endosomal transport, lysosomal enzyme targeting and autophagy [2]. An important group of lysosomal membrane proteins are the vacuolar-type proton ATPase complexes, which pump protons into the lysosomes to maintain the low luminal pH necessary for optimal hydrolase activity [3]. Inward chloride channels in the lysosomal membrane neutralize the resulting increase in positive charge [3]. At least in mammals, most of the hydrolytic enzymes are transported to the lysosomes via the 'direct' mannose-phosphate pathway, while lysosomal membrane proteins use both the 'direct' pathway and the 'indirect' pathway via the plasma membrane, to travel to the lysosome [4, 5]. No homologs of mannose-6-phosphate receptors have been identified in *C. elegans*, suggesting that mannose-6-phosphate signals for the sorting of newly synthesized lysosomal enzymes may have been replaced by an amino acid based signals and a vps10/sortilin receptor system similar to that in yeast [6]. Furthermore, although the post-translational modification pathways are conserved, the structures of the glycoconjugates (glycolipids, O-glycans and N-glycans) in mammals are different from those in flies and worms. Worms have phosphorylcholine substituted glycolipids and do not have sialic acid modifications [7, 8, 9].

Lysosomes obtain their constituents and material to be degraded through vesicular trafficking. For example, transport vesicles containing hydrolytic enzymes can combine with lysosomes or vesicles filled with endocytosed compounds called endosomes. After the endocytosed materials have been degraded, lysosomal proteins can be recycled, catabolized materials can be reused as building blocks for the anabolic processes in the cell, and waste products may be excreted from the cell. Furthermore, similar processes of vesicular fission or fusion, and intralysosomal

degradation may occur with autophagosomes in order to recycle old or obsolete organelles or other parts of the cell.

### **Lysosomal storage diseases**

When any process discussed above is not working properly, this may lead to partly or completely dysfunctional lysosomes that are unable to degrade specific compounds causing their accumulation (See [10] for a detailed description of lysosomal storage diseases). The mass of stored materials can cause the lysosomes to become inflexible or enlarged cellular compartments that are disrupting other processes taking place in the cell, thus leading to a lysosomal storage disorder. Most of the lysosomal storage diseases are caused by defects in hydrolytic enzymes, such as the acid  $\alpha$ -glucosidase deficiency in the first described lysosomal storage disorder, Pompe disease (MIM232300)[11]. Acid  $\alpha$ -glucosidase deficiency causes storage of glycogen in lysosomes of numerous tissues. In the most severe form of this disease, patients suffer from prominent cardiomegaly, hypotonia, hepatomegaly and they finally die due to cardiorespiratory failure, usually before the age of two [12].

In addition to enzyme deficiencies, disturbed protein sorting or vesicular trafficking may also lead to lysosomal storage disorders, e.g., in mucopolidosis type II or I-cell disease (MIM252500). Cells of mesenchymal origin from I-cell disease patients have reduced N-acetylglucosamine-1-phosphotransferase activity in the Golgi apparatus and fail to add an N-acetylglucosamine-1-phosphate group to the mannose residues already present on the lysosomal enzyme precursors [13]. Lysosomal enzymes lacking proper modification are secreted instead of normally transported to the lysosomes. Affected cells contain dense inclusions of storage material, hence the name inclusion cell or I-cell disease. I-cell disease patients generally suffer from

severe progressive psychomotor retardation and premature death in the first decade of life.

The severe pathologies of some lysosomal storage disorders have prompted research into the etiology of the more than 40 known lysosomal storage diseases [14, 15]. For most diseases causative mutations have been described and possible treatments are being developed. For instance, enzyme replacement therapy provides treatment of Gaucher's disease, in which the gene encoding the enzyme acid  $\beta$ -glucosidase is mutated [16]. For most lysosomal storage diseases only symptomatic treatment of the patients is possible at the moment. Although much is known concerning lysosomal processes and the mechanisms required for a lysosome to be functional, some questions remain unanswered: whether and how undigested accumulated materials cause the symptoms observed in patients suffering from lysosomal storage diseases or whether the accumulation is a primary or merely a side effect and why only certain cell types are affected [17].

#### **Prerequisites of lysosomal disease models and *C. elegans***

To investigate the mechanisms underlying lysosomal storage disorders and their relation with the disease phenotype, appropriate model organisms are required. These organisms preferably should be eukaryotic, multicellular organisms, but straightforward to use and genetically easily modifiable. Furthermore, a fully sequenced genome would help to identify homologous genes and well-characterized developmental pathways would facilitate the study of mutant phenotypes. This profile is matched by the mouse, the fly *Drosophila melanogaster* and the less complex nematode *Caenorhabditis elegans*, which was initially described by Sydney Brenner [18]. The nematode is a convenient and nowadays widely used model organism with a 3-day generation time and an entirely known cell lineage. These worms exist in two

sexes: hermaphrodites that easily self-fertilize to obtain homozygous mutants and males that can be used to perform crosses. Moreover, since neurological symptoms are common in human lysosomal storage diseases, the very well characterized and invariably wired nervous system of *C. elegans* might be an advantage. The hermaphrodite has 302 neurons on a total of 959 cells. The worm is amenable to large genetic screens and a comprehensive toolset for worm research on a genetic, cellular, and behavioral level is available. Gene specific loss-of-function mutations can easily be phenocopied using RNA interference (RNAi) by microinjection, soaking or feeding methods. Whole genome approaches have delivered a vast amount of data, even on previously unannotated sequences, providing clues to the function of the putative proteins [19]. In the context of lysosomal research, it should be pointed out, however, that *C. elegans* genetics has evolved much more than its cell biology and biochemistry.

### **Lysosomes in *C. elegans***

Clokey and Jacobsen [20] were the first to hypothesize that the gut granules, which fill the intestinal cells of *C. elegans*, were intestine-specific secondary lysosomes, which are formed by the fusion of a primary lysosome and a vesicle containing material to be degraded, such as a phagosome or pinosome. The gut granules are filled with birefringent material and easily detected in living worms. The lysosomal markers Acridine Orange and LysoTracker Red mainly stain the gut granules suggesting that they are related to lysosomes. In addition, immunostaining of homologues to known lysosomal proteins indicates their colocalization with gut granules. The stainings also suggest that lysosomes or lysosome-related organelles are not easily detectable with these markers in most other cell types of wild type worms. Transgenic worms

expressing lysosomal protein-GFP fusions have been constructed to determine the expression pattern of lysosomal genes [21]. The drawback of using such transgenic worms for organelle detection is that the fusion proteins may affect normal lysosomal function. Three populations of vesicles with different electron densities are visible in gut cells by electron microscopy [22]. Lysosomes are not readily observed in other cells, apart from coelomocytes, scavenger cells, which use endocytosis to take up materials from the body cavity of the worm before lysosomal degradation [23]. Therefore, studies on *C. elegans* lysosomes have focussed on the formation of gut granules and the uptake of material by coelomocytes, which have been regarded as equivalents of lysosomal biogenesis and lysosomal degradation. Successful screens were performed for mutants showing gut granule loss (*glo*) or coelomocyte uptake defects (*cup*) to identify candidate genes involved in these lysosomal processes [23, 24].

Therefore, we have investigated the potential of this worm as a model organism for lysosomal storage disorders.

We have compared all human protein sequences known to be involved in lysosomal storage disorders with *C. elegans* protein sequences using the best reciprocal Blast score to identify the putative worm homologues of these lysosomal disease proteins.

Subsequently, we compiled all phenotypes of nematodes with mutations in the putative homologous genes in order to get an overview of all possible lysosomal phenotypes present in the worm. Similar comparisons were performed with human proteins that were involved in lysosomal processes but that were still unassociated with disease. The homologues and their phenotypes were listed, as were those of the already known *C. elegans* loci involved in lysosomal function, encompassing all possible lysosomal phenotypes that are known in *C. elegans* at the moment. The

suitability of these lysosomal phenotypes for genetic screens was studied to discuss their potential to identify modifying mutations in genes involved in the same molecular process as the *C. elegans* homologues.

ACCEPTED MANUSCRIPT

***C. elegans* homologues to human lysosomal storage disease genes**

We identified *C. elegans* homologues for almost all 58 human genes associated with lysosomal storage diseases (Table 1). Thirty three of the human genes encode lysosomal enzymes, but only 21 enzyme encoding homologues were present in the worm. Unfortunately, most enzyme activities have not been determined in relation to specific genes due to lack of mutants or redundancy. Most of the human disease genes have only a single homologue, except the two involved in Niemann-Pick disease, *NPC1* and *SMPD1* (two and three homologues, respectively), and the juvenile neuronal ceroid lipofuscinosis gene *CLN3* (three homologues). Conversely, a single worm homologue has been identified for each of three human gene pairs, *HEXA-HEXB*, *GLA-NAGA* and *GALNS-ARSA*. For all 42 *C. elegans* homologues, information on gene function can be found in literature and in the Wormbase database [25]. Ten of these *C. elegans* genes, which have been studied individually in the nematode, are homologous to genes involved in mucopolidosis type IV (MIM252650), Niemann-Pick type C (MIM257220), Danon disease (MIM300257), Hermansky Pudlak Syndrome-2 (MIM608233) and congenital, infantile, and juvenile forms of neuronal ceroid lipofuscinosis (NCL) (MIM610127, MIM256730, and MIM204200, respectively). Data on the other 32 genes were derived from sequence similarities or came from whole genome approaches, such as microarray analyses [26], and whole genome RNAi experiments [27], of which the details are listed in Wormbase. After RNAi knockdown of 35 genes, worm phenotypes ranged from none (26 times) to (embryonic) lethality (twice) with seven subtle intermediary phenotypes (Table 1). Although mutants have been isolated for 19 of the genes, 7 out of the 11 mutants characterized had a mainly subtle phenotype. The available information about

nematode homologues to human lysosomal storage disease genes is summarized below in order to get an overview of all possible phenotypes of worm models for lysosomal storage disorders.

*C. elegans cup-5*, homologous to the Mucopolidosis type IV gene *MCOLN1*, and lysosome biogenesis

Mucopolidosis type IV, caused by mutations in the *MCOLN1* gene, is a neurodegenerative lysosomal storage disorder of which the main symptoms are psychomotor retardation and ophthalmologic abnormalities [28-30]. Ultrastructural analysis of patient tissues usually reveals many enlarged vacuoles, presumably lysosomes in which lipids are stored as well as water-soluble granulated substances. The *MCOLN1* gene encodes the lysosomal membrane protein h-mucopolin-1, which has six predicted transmembrane domains and functions as a non-selective cation channel of which the activity is modulated by pH [31]. The *C. elegans MCOLN1* homologue *cup-5* is a functional orthologue of the human *MCOLN1* gene, since the coelomocyte uptake (Cup) phenotype of *cup-5* mutants, consisting of heterogeneous enlarged vacuoles and embryonic lethality, can be rescued by expressing the human gene in these mutants [32]. Mutations in the *cup-5* gene were identified by screening for mutants with disrupted endocytosis [33], and in mutants with affected programmed cell death [32]. The *cup-5* gene is expressed in most tissues in adult worms, and subcellularly localized to nascent and mature lysosomes [34]. Similar to patient cells, cells from *cup-5* mutants have enlarged vacuoles and lysosomes, apparently due to defective lysosomal degradation processes. The CUP-5 protein was suggested to play a role in lysosome biogenesis or maturation [34], because h-mucopolin-1 was shown to be a Ca<sup>2+</sup>-permeable channel [35], and Ca<sup>2+</sup> transport is essential for fusion of late endosomes and lysosomes and for reformation of

lysosomes [36]. Interestingly, through a screen for modifier alleles, a mutation in the *mrp-4* gene, encoding an endosomal-lysosomal ABC transporter, was shown to suppress *cup-5* lethality and rescue the lysosomal degradation and developmental defects of the *cup-5* mutants [37]. In the *cup-5* mutants, degradation of the ABC transporter was delayed, causing an imbalance in the endosomal-lysosomal import of compounds, which probably interferes with normal degradation processes. The affected degradation is thought to lead to starvation of the cells and independently to developmental defects. Absence of the ABC transporter results in rescue of lysosomal function, thereby permitting the cells to survive. Whether similar events contribute to the cellular and neuronal degeneration in Mucopolipidosis type IV patients is still unknown, but if this is the case reducing the activity of ABC transporters might provide for a therapy for the treatment of mucopolipidosis type IV.

#### Nematode Niemann-Pick type C homologues involved in cholesterol trafficking

Multiple forms of Niemann-Pick disease exist, either caused by deficient acid sphingomyelinase activity leading to accumulation of sphingomyelin in Niemann-Pick type A and B [38, 39], or by defective cholesterol trafficking resulting in lysosomal storage of unesterified cholesterol in Niemann-Pick type C [40]. Symptoms caused by acid sphingomyelinase deficiency vary from hepatosplenomegaly and progressive neurodegenerative disease to pulmonary infiltration. Patients suffering from defective cholesterol trafficking also display progressive neurological disease and possibly prominent hepatic damage. Three acid sphingomyelinase (ASM) homologues were identified in *C. elegans*, while in other organisms only one ASM could be identified [41]. Unfortunately, according to the Wormbase database, mutants for only one of the *C. elegans* ASM homologues are available, but their phenotypes

were not described. No *ASM* RNAi phenotypes emerged from genome-wide RNAi screens [25], but RNAi targeted against multiple *ASM* genes perhaps could result in an interesting knock-down phenotype and provide a model for Niemann-Pick type A and B.

Niemann-Pick type C can be caused by mutations in two genes, *NPC1* and *NPC2*, encoding proteins with NPC and sterol sensing domains that are implicated in retrograde transport from sterols and other cargo from lysosomes [40, 42, 43]. Each has one worm homologue, *ncr-1* and *ncr-2*, respectively [36]. Worms without *ncr-1* are hypersensitive to lack of cholesterol, an essential substance for the nematode, and to exposure to progesterone, which can inhibit intracellular cholesterol trafficking in mammalian cells [44, 45]. In contrast, *ncr-2* single mutants appear superficially wild type. Whereas *ncr-2; ncr-1* double mutants display constitutive inappropriate dauer formation, which could be rescued by microinjection of NCR-2 or NCR-1 wildtype genes, suggesting both proteins play redundant roles to prevent dauer formation under favorable conditions. The dauer is a relatively stress-resistant alternative larval life stage that the animal can form when it develops under stressful conditions.

Furthermore, *ncr-2; ncr-1* double mutants have abnormal morphology of certain neurons during transient dauer stage. Other *ncr-2; ncr-1* double mutant phenotypes encompass developmental pleiotropic phenotypes similar to *daf-9* and *daf-12* mutants including reproductive defects, concordant reduced brood size and life span, vulval abnormalities and disruption of the cuticular alae. Epistasis analysis placed NCR-1 and NCR-2 upstream of DAF-9 in the dauer formation pathway [44, 45]. Therefore, the NCR-1 and NCR-2 proteins that were suggested to play a role in intracellular cholesterol trafficking may provide the substrate for DAF-9, the ER localized cytochrome P450 enzyme, which catalyzes a reaction to form a lipophilic hormone

for the DAF-12 nuclear receptor [46, 47]. Under cholesterol-deprived conditions, *ncr-2*; *ncr-1* double mutant worms could be unable to efficiently transport this hormone progenitor to the site of DAF-9 action and hence stimulate dauer formation due to absence of the signal to bypass the dauer stage. Further examination of how the mutations in *NCR-1* and *NCR-2* lead to the other phenotypes, and, most interestingly, the abnormal neuronal morphology, may elucidate other functional aspects of these proteins.

*C. elegans* LMP-1 protein, homologous to the Danon disease protein LAMP2, involved in lysosome biogenesis

Mutations in the lysosome-associated membrane protein-2 gene (*LAMP2*) cause glycogen storage disease type IIb or Danon disease, characterized by cardiomyopathy, myopathy and variable mental retardation [48]. Originally, Danon disease was described as a variant glycogen storage disease type II, since acid-maltase or alpha-glucosidase activity was normal [49]. *LAMP2* and structurally similar *LAMP1* are heavily glycosylated lysosomal membrane proteins with one transmembrane domain and a major intralysosomal part, and are thought to be functional in lysosome stability and integrity [2]. *LAMP-1* and *LAMP-2* knock-out mice have been generated to investigate *LAMP* protein function [50, 51]. *LAMP-2* knock-out mice suffered from cardiomyopathy and accumulation of autophagic vacuoles, similar to human Danon disease patients. *LAMP-1* knock-out mice displayed no overt phenotype but overexpressed *LAMP-2*, suggesting functional redundancy. Additional functional and morphological studies of the heart of *LAMP-2* knock-out mice showed that these mice suffered from contractile dysfunction that was suggested to be due to morphological changes [52]. Embryonic fibroblasts from *LAMP-1 LAMP-2* double knockout mice show impaired motility and fusion of lysosomes to phagosomes, which

suggests that the LAMP proteins are involved in lysosomal fusion to phagosomes and may explain the accumulation of autophagic vacuoles [53]. Kostich and coworkers [22] have searched the *C. elegans* genome for LAMP-like sequences and identified a LAMP homologue *lmp-1*, which has a lysosomal targeting sequence (GYXX $\Phi$ , in which  $\Phi$  is a large hydrophobic amino acid residue and X an unspecified amino acid) at its COOH terminus. Our BLAST search with the human LAMP2 protein sequence against the *C. elegans* protein database Wormpep [25] resulted in the highest hit, with LMP-1 and a lower score with LMP-2, which has no GYXX $\Phi$  motif. Nematode *lmp-1* deletion mutants are viable and fertile, show alternative intestinal granule populations, and apparent loss of one type of granule, hence *LMP-1* is likely to be functional in lysosome biogenesis or maintenance [22]. How loss of the *lmp-1* gene causes this change in granule composition and whether the irregular granules reminiscent of granule fusion or engulfment indicate that autophagy is affected in these mutants remains to be elucidated. If *lmp-1* gut granule loss can be scored under a standard Differential Interference Contrast (DIC) microscope, screening for mutations that resemble or modify the *Lmp-1* phenotype could be feasible [54].

#### Hermansky Pudlak syndrome type 2 (HPS type 2) and the AP $\beta$ 3a homologue in the worm implicated in development

The heterogenous group of diseases collectively known as Hermansky-Pudlak syndrome (HPS) is pathologically characterized by prolonged bleeding, albinism and lysosomal storage of ceroid, and presumably results from defects in multiple cytoplasmic organelles, such as melanosomes, platelet-dense granules, and lysosomes [55]. Mutations in eight genes, *HPS1* – *HPS8*, have been shown to cause this disorder and the proteins encoded by these genes are thought to be involved in the biogenesis or transport of lysosomes or lysosome related organelles [56, 57]. The HPS-1 and

HPS-4 proteins appear to form a complex that might play a role in the biogenesis of lysosomes [58, 59]. These proteins were suggested to function independently from the Adaptor Protein 3 (AP-3) complex, which is involved in formation of carrier vesicles and cargo recruitment for protein transport. We could not identify *C. elegans* sequences homologous to HPS-1, HPS-3, HPS-4, and HPS-6 by mere protein sequence comparison, suggesting that these proteins are simply not present in the nematode or their sequences have diverged beyond recognition. HPS-5, HPS-7 and HPS-8 have putative nematode homologues, but these have not yet been investigated individually and the high throughput approaches did not elucidate any functional aspects of these proteins [25]. HPS type 2 is caused by mutations in the gene encoding the  $\beta$ 3a subunit of the AP-3 complex [60]. AP-3 is involved in the sorting of transmembrane proteins from endosomes and the *trans*-Golgi network to lysosomes and endosome-lysosome related organelles, but it is unknown how altered AP-3 function leads to HPS type 2 [61, 62]. The *C. elegans* homologue for this protein, *Apb-3*, appears to be required for development as RNAi knock-down of the *apb-3* transcript causes embryonic and larval lethality [63]. Interestingly, worm knock-outs for two other AP-3 complex subunits, *apt-6* and *apt-7*, encoding  $\beta$ 3 and  $\mu$ 3 subunits respectively, have an embryonic gut granule loss (Glo) phenotype and larvae and adult mutant worms have less autofluorescent gut granules [24]. The autofluorescent gut granules are presumed to be secondary lysosomes that may contain yolk or other nutrients [20, 22]. Loss of gut granules due to affected *Apb-3* function may indicate a reduced capacity to degrade materials taken up by endocytosis or phagocytosis to nutrients, leading to the nutrient deprivation and subsequent lethality observed in *apb-3* RNAi worms. Additional investigations, such as genetic screens for alleles

modifying the larval arrest or the Glo phenotypes, could provide further insight into AP-3 dependent processes and the precise role of AP-3 in protein trafficking.

Neuronal ceroid lipofuscinoses (NCL) and the worm *asp-4*, *ppt-1* and *cln-3* homologues

The congenital, infantile and juvenile forms of NCL are caused by mutations in the *CTSD*, *PPT1* and *CLN3* genes respectively, which all lead to severe neurodegenerative disorders with similar disease progression but with different age of onset [64-66]. Initial symptoms include visual deterioration followed by epileptic seizures, progressing to a state of dementia and ending in premature death [15]. In addition to the differences between the age of onset and the genes affected, the NCLs can also be distinguished by the typical patterns of the lipopigment accumulations found in lysosomes of neurons and other cell types [67]. No direct links have been established between the causative mutations, the observed pathology and the accumulated material. The most severe form of the NCLs, congenital neuronal ceroid lipofuscinosis, is very rare and thus far only 10 patients have been described [64]. Patients suffering from this disease are microcephalic, may have seizures and die soon after birth. This disease is caused by mutations in the *CTSD* gene encoding cathepsin D and strongly resembles the congenital ovine neuronal ceroid lipofuscinosis previously identified in sheep [68]. The nematode homologue of the *CTSD* gene *asp-4* is also the closest homologue of the human *CTSE* gene, which is mutated in CTSE deficiency, a disease distinct from NCL [69]. *Asp-4* was shown to be involved in necrotic cell death as *asp-4* RNAi knockdown leads to decreased cell death, signifying its role as an executioner protease [70]. Thus, altered cell death may underlie the etiology of congenital neuronal ceroid lipofuscinosis.

PPT1 encodes the lysosomal enzyme palmitoyl protein thioesterase-1, which cleaves thioester linkages in S-acylated (palmitoylated) proteins and facilitates the removal of the palmitate residues [71]. In neurons however, PPT1 is also found in non-lysosomal compartments, synaptic vesicles and synaptosomes [72]. The *C. elegans* *CLN1* homologue is designated *ppt-1* and worms mutated in their *ppt-1* gene display mitochondrial abnormalities at an ultrastructural level, and an incompletely penetrant reproductive phenotype, so-called ‘bagging’ where eggs hatch inside the parent [73]. The juvenile NCL gene *CLN3* encodes a transmembrane protein, which is thought to be primarily localized to lysosomes and may be implicated in pH regulation or amino acid transport [67]. The nematode has three homologous genes, *cln-3.1*, *cln-3.2*, and *cln-3.3*, which when mutated and combined into one triple mutant strain causes a mild decrease in life span and brood size [74]. The phenotypes of the *ppt-1* and *cln-3* mutants are not very suitable for genetic screens without additional enhancement of the phenotypes. This might be accomplished by adding compounds interfering with lysosomal acidification. Although the latter may not be very specific, it might be sufficient to tip the balance in favor of the more robust phenotype without increasing its frequency in the wildtype population.

#### ***C. elegans* homologues to human genes associated with lysosomal function involved in other disorders**

Not all mutations in genes associated with lysosomal function cause lysosomal storage disease. The reason may be that these genes encode proteins, which are localized in lysosomes or lysosome-related organelles in certain cell types but at a different location in others. The known mutations in these genes may impair the non-lysosomal function of the proteins more than the lysosomal one. We have compiled a list of thirteen disease genes encoding proteins without clear lysosomal localization in the most affected cell types or organs and their worm homologues (Table 2). Three of

these genes, *CTSC*, *MPO* and *TCIRG1* belong to gene families with multiple homologues in the worm. Remarkably, most of these genes seem to be associated with defects in polarized cells, such as renal tubular cells in renal acidosis or osteoclasts in osteopetrosis. About half of their proteins are localized in the plasma membrane. Many are subunits of the conserved vacuolar ATPase pumping protons through the plasma, lysosomal and other organellar membranes, but *CLCN7* is a channel for chloride ions compensating the positive charges of the protons in vesicles. Several proteins are hydrolytic enzymes, such as *CTSC* and *CTSK*, involved in degradation processes in lysosome-related organelles. One of these processes is bone resorption, which is performed by specialized cells called osteoclasts [75]. On contact with bone, part of their membrane can form a ruffled border and create a resorptive pit, which is acidified and filled with hydrolytic enzymes by lysosomes to degrade bone. Defects in the acidification or the hydrolytic enzymes can prevent bone resorption leading to osteopetrosis. Bone defects similar to those in osteopetrosis have been observed in mucopolysaccharidosis type VII and have also been attributed to malfunctioning osteoclasts [76]. Thus, with the environment of the resorptive pit resembling that of a lysosome, one might look at osteopetrosis as a kind of extracellular lysosomal disorder. The four remaining genes, *OCRL1*, *PIP5K3*, *VPS13A*, and *VPS33B* play a role in lysosomal protein trafficking and lysosomal maturation. Mutant and/or RNAi knockdown phenotypes have been determined for all 13 genes, but no obvious lysosomal phenotype was observed apart from the enlarged lysosomes in mutants of the worm *PIP5K3* homologue, *ppk-3*, with their lysosomal maturation defect [77]. Although no mutations leading to lysosomal storage has been identified yet, the human genes listed in Table 2 could be regarded as candidate genes for lysosomal storage disorders, since knockout mouse models of *CLCN7* and

OSTM1 display lysosomal storage and neurodegeneration in addition to osteopetrosis [78, 79].

### ***C. elegans* homologues to human genes involved in lysosomal functioning**

To complement the collection of potential lysosomal phenotypes of the worm, we have generated a list of 92 human lysosomal genes not yet implicated in disease and searched for their worm homologues (Table 3).

At least one worm homologue was found for each of the 84 human lysosomal genes.

In some cases, for example vacuolar ATPase subunit genes, the same worm homologue was found for several human genes or vice versa, indicating that these genes have been duplicated or lost in one organism compared to the other. In five other cases, a human gene family with many members, such as the cathepsin and lectin families, is also represented in the worm

For each of the 94 worm homologues listed, we have compiled the available information about their mutant and RNAi knockdown phenotypes. Mutants for 40 homologues have been identified, but only half of them have been characterized phenotypically. Six of these have no obvious phenotype. Most of the homologues have been included in genome-wide RNAi knockdown experiments, but for 57 of them no phenotype was observed. The most common phenotype observed in RNAi knockdown experiments for these genes was embryonic lethality (Emb: 28 times) followed by lethality (Let: 22 times) and larval arrest (Lva: 15 times). A selection of these homologues with interesting phenotypes is discussed below.

#### The *C. elegans* LRP-2/glycoprotein 330 homologue is essential for nematode life

LRP2/glycoprotein 330 is a member of the LDL receptor protein (LRP) family involved in endocytosis. Its mouse, zebrafish and worm homologues play a role in

development [80]. The *C. elegans* homologue of this protein, *lrp-1* was shown to be essential for growth and development of the nematode, as *lrp-1* mutants show larval arrest and molting defects [81]. Moreover, homozygous *lrp-1* mutants also are moderately dumpy, a phenotype observed previously in worms with cuticle synthesis deficiencies [82]. Although *lrp-1* mutants appeared to have normal cuticle synthesis, they may have aberrant cuticle renewal. Interestingly, *lrp-1* mutants phenocopied wildtype worms that were starved for cholesterol, supporting a role for LRP-1 in sterol endocytosis. Recently, a genetic interaction was identified between LRP-1 and *hgrs-1*, which is involved in sorting of endocytosed proteins [83]. In *hgrs-1* mutants, the LRP-1 protein was mislocalized, indicating that *hgrs-1* is required for correct endocytic trafficking of the LRP-1 protein. The phenotype of *hgrs-1* mutants resembled that of *lrp-1* mutants and wildtype worms starved for cholesterol. This suggests that other genetic or biochemical interactions may be identified by isolating mutants with *lrp-1*-like phenotypes or through RNAi screens for modifiers of the *lrp-1* phenotype.

#### The multifunctional role of cathepsin Z in nematode development

The cathepsin Z protein is a cysteine protease that acts as a carboxymonopeptidase and is incorporated in the phagosome [84]. The protein was detected in cells of the immune system and in tumor cells and was suggested to also play a role in cell adhesion dependent on  $\beta_2$ -integrin [85]. The nematode homologue of this protein, CPZ-1, was shown to be essential for development as RNAi knockdown and mutation of *cpz-1* both lead to embryonic or larval lethality in part of the worm population [86]. Whether this developmental arrest is caused by defective phagosome function or cell adhesion or has another cause remains to be elucidated. The exact function of CPZ-1

could be further investigated in *cpz-1* mutants or worms depleted for CPZ-1 by RNAi knockdown.

#### A *C. elegans* chloride channel involved in endocytosis

The chloride channel protein CLC-3 may play a role in the stabilization of membrane potential of intracellular organelles, and also in transepithelial transport, cell volume regulation, and endocytosis [87]. Interestingly, deletion of the homologous mouse gene, *Clc3*, causes hippocampal neurodegeneration and retinal degeneration, probably by toxic glutamate concentrations in the synaptic vesicles of neurons or defective acidification in the endosomal or recycling pathways of these cells [88]. Similar to mammals, *C. elegans* has multiple chloride channels, including the ubiquitously expressed CLC-3 homologue, *CLH-5*, which seems to be involved in receptor mediated endocytosis, because *clh-5* RNAi caused an endocytosis defect similar to the *cup-5* mutant [23]. The relatively mild *clh-5* RNAi phenotype could be explained by chloride channel redundancy.

#### **Human genes involved in lysosomal functioning without *C. elegans* homologues**

Eight of the human genes listed in Table 3 have no worm counterpart based on protein sequence similarity. Four of them encode subunits of the biogenesis of lysosome-related organelles complex 1 (BLOC1), of which eight subunits have been identified in mammals [89]. The worm has only homologues of one BLOC1 subunit involved in Hermansky-Pudlak syndrome, DTNBP1, and the BLOC1S1, BLOC1S2, and SNAPAP subunits, which are not associated with human disease. It is unclear whether these proteins are part of a conserved BLOC1 core complex that on its own can play a role in organelle biosynthesis, or whether they interact with unidentified partners, or have acquired a different function. Another gene, PCYOX1, encodes an enzyme

involved in the degradation of prenylcysteines [90]. Although knockout mice accumulate prenylcysteines in brain cells, this does not seem to lead to lysosomal storage, brain pathology or histological features normally associated with lysosomal storage diseases. It is tempting to speculate that the accumulating prenylcysteines can be localized in (lysosomal) membranes without problems until their concentration starts to destabilize these membranes or the altered membrane composition interferes with the function of membrane proteins.

### **Comparison of phenotypes related to *C. elegans* loci affecting lysosomal function**

The size, number, appearance or electron density of the gut granules or other lysosome-related vesicles in coelomocytes have been used in genetic screens to isolate mutants with putative lysosomal phenotypes, such as coelomocyte uptake (Cup) and gut granule loss (Glo). Genetic mapping has led to the identification of several genes involved in these phenotypes, which have been compiled together with other potential lysosomal phenotypes in Table 4. The loci *cup-2*, *cup-4*, *glo-4* and *glo-5* were found to encode homologues to *DERL1*, *CHRNB1*, *HERC4* and *ABCB1*, respectively [23, 24, 91]. In addition, three existing *rme* mutants displayed the Cup phenotype [23], whereas five known genes were involved in the Glo phenotype [24](Table 4). These genes encode proteins with functions in other locations within the cell. Many of these proteins probably play an important role in the processes discussed in the introduction: lysosome biogenesis, maintenance of endosomal transport and lysosomal enzyme targeting. The associated phenotypes vary in severity, but are relatively subtle and more similar to those in Table 1 than to those in Tables 2 and 3.

**SUMMARY and CONCLUSION**

Most of the human genes involved in lysosomal storage disorders have single *C. elegans* homologues (Table 1). A few human genes have more than one worm counterpart and vice versa. The reason for the existence of multiple worm homologues of *SMPD1* and *CLN3* is unclear, although temporal and spatial expression patterns differ for the *asm* and *cln-3* paralogues [41, 74]. The representation of three human enzyme-encoding gene pairs, *HEXA-HEXB*, *GLA-NAGA* and *GALNS-ARSA*, by single worm homologues might be considered as an indication of simpler biochemical pathways in the worm. The worm GANA-1 homologue combines the *GLA* and *NAGA* enzyme activities into one multifunctional enzyme [92]. Several genes (e.g., 6 *HPS* and 4 *CLN* genes) involved in human lysosomal storage disorders have no worm counterparts, which may in part be associated with the lack of cell types with specialized lysosome-related cell organelles, such as melanosomes, in the worm. This may also explain why the worm lacks four subunits of the *BLOC1* complex involved in the biogenesis of lysosome-related organelles (Table 3). Furthermore, due the complexity of the human brain, man may require a larger repertoire of *CLN* proteins, some of which have additional roles in synaptic vesicles. In general, however, the nematode *C. elegans* has largely the same number of proteins involved in lysosomal processes as man.

The mutant or RNAi knockdown phenotypes for 147 worm genes ranged from embryonic lethality via subtle intermediate phenotypes to none observed (Tables 1-3). Phenotypes of lysosomal storage disease related worm homologues (Table 1) or lysosomal worm mutants (Table 4) seem to be more subtle than those of homologues, which are not yet associated with lysosomal storage disease (Table 3). Worm models of human lysosomal storage disorders have diverse phenotypes ranging from

seemingly apparent lysosomal nature, such as the endocytosis defect in *cup-5* mutants or *Imp-1* mutants that have lost one type of intestinal granule presumed to be pre-lysosomes, to less directly lysosome-related, such as the disturbed cholesterol trafficking of the *ncr* mutants, developmental arrest or decreased life span or brood size. Specific phenotypes associated with changes in lysosomal proteins can also be caused by defects in proteins, which do not localize to lysosomes, but exert their function in lysosome biogenesis, maintenance of endosomal transport and lysosomal protein targeting. Their human homologues might be good candidates for disorders with lysosomal storage or lysosomal dysfunction without known cause.

Severe phenotypes, such as (embryonic) lethality and larval arrest, are observed for genes encoding parts of the vacuolar proton pump. The latter genes play an important role in multiple processes and organelles. Their inactivation may lead to pleiotropic effects, having a more severe impact on the worm. Multiple copies of the vacuolar proton pump genes in man, however, may provide sufficient redundancy to prevent severe pleiotropic effects unless multiple genes are hit.

Genetic redundancy or the life span of the worm, which may be too short for lysosomal storage to occur, could explain the absence of a mutant and RNAi knockdown phenotype for many nematode homologues. In addition, most of the mutants were tested under standard laboratory conditions, which are presumed to be ideal, evading any stress required for the manifestation of a phenotype.

Furthermore, even when a knock-out mutation causes a phenotype, RNAi knock-down will not always result in a phenotype [93]. Last but not least, regular genome-wide RNAi screens yield a mass of data potentially providing a starting point for the investigation of any individual gene, but present the researcher with the risk to overlook certain subtle phenotypes. Although the Cup and Glo phenotypes are

relatively easy to score using fluorescence microscopy [23, 24], these were not scored in the genome-wide RNAi screens. Determining a difference in number, size or electron density of the lysosomal vesicles (Lmp-1, Idi phenotypes) however, requires time-consuming ultrastructural investigations, which are usually not included in screens. In general, biochemical parameters (enzyme activity, metabolic measurements) are also not measured.

The identification of additional worm mutants by genetic screens would help to identify new players in the different processes necessary for normal lysosomal function and potential new candidates for human hereditary disorders. The nature of the mutant, genetic or RNAi knockdown, also determines the screenability of modifier genes as well as the penetrance of the phenotype. The advantage of the most observed worm phenotypes in Table 3, developmental arrest or lethality in any life stage, is the relative ease with which genetic screens can be done for modifiers of these robust phenotypes. Although many lysosomal storage diseases affect the human brain, the corresponding worm models did not display lysosomal storage or other effects in the well-characterized nervous system, thereby eliminating one of the advantages of the worm compared to other model organisms. Whether the worm is a suitable model organism to investigate lysosomal storage diseases depends for a large part on the presence of a clear and sufficiently penetrant phenotype. The observation of many subtle lysosomal mutant phenotypes in the worm, however, suggests that it may not be trivial to perform screens for new mutants.

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**Table 1. Human Lysosomal Storage Disorder Genes and their *C. elegans* Homologues**

Lysosomal storage disorder	Human Gene	MIM number (a)	<i>C. elegans</i> Homologue	% Similarity to Human Gene	Function of human protein	<i>C. elegans</i> Mutant (c)	RNAi and phenotypes (e)
<b>Neuronal ceroid lipofuscinoses (NCL)</b>							
Infantile NCL	<i>PPT1</i> ( <i>CLN1</i> )	600722	F44C4.5	54	Palmitoyl-protein thioesterase 1 precursor (EC 3.1.2.22)	<i>ppt-1</i>	M: delayed egg-laying, abnormal mitochondria; R: None observed
(Congenital ovine NCL)	<i>CTSD</i>	116840	R12H7.2b)	61	Cathepsin D precursor (EC 3.4.23.5)	<i>asp-4 d)</i>	R: Ced
Late infantile NCL	<i>TPP1</i> ( <i>CLN2</i> )	204500	None		Tripeptidyl-peptidase 1 precursor (EC 3.4.14.9)		
Juvenile NCL	<i>CLN3</i>	204200	F07B10.1 C01G8.2	53 49	Unknown	<i>cln-3.1</i> <i>cln-3.2</i>	M: slightly reduced life span; R: None observed M: slightly

Late infantile NCL, Finnish variant	<i>CLN5</i> 256731	None	ZC190.1	62	Unknown	<i>cln-3.3</i>	reduced brood size; R: None observed
Late infantile NCL, Indian variant	<i>CLN6</i> 601780	None			Unknown		R: None observed
Northern epilepsy	<i>CLN8</i> 600143	None			Unknown		
<b>Oligosaccharidoses</b>							
Alpha-mannosidosis	<i>MAN2</i> <i>BI</i> 248500		F55D10.1	52	Lysosomal alpha-mannosidase precursor (EC 3.2.1.24)		R: None observed
Beta-mannosidosis	<i>MANB</i> <i>A</i> 248510		C33G3.4	53	Beta-mannosidase precursor (EC 3.2.1.25)		R: None observed
Fucosidosis	<i>FUCA</i> <i>I</i> 230000		W03G11.3	50	Tissue alpha-L-fucosidase precursor (EC 3.2.1.51)		R: None observed
Farber's disease	<i>ASAH</i> <i>I</i> 228000		K11D2.2	62	Acid ceramidase precursor (EC 3.5.1.23)	<i>asah-1 d)</i>	R: Age, Reduced lifespan
Aspartylglucosaminuria	<i>AGA</i> 208400		R04B3.2	56	N(4)-(beta-N-acetylglucosaminy)-L- asparaginase precursor (EC 3.5.1.26)		R: None observed
Galactosialidosis	<i>PPGB</i> 256540		F41C3.5	59	Lysosomal protective protein precursor (EC 3.4.16.5)		R: None observed
<b>Sphingolipidoses</b>							
Tay-Sachs disease,	<i>HEXA</i> 272800		T14F9.3	56	Beta-hexosaminidase alpha chain	<i>hex-1</i>	M: No

GM2 gangliosidosis I				precursor (EC 3.2.1.52)		chitotriosidase activity, reduced hexosaminidase activity; R: None observed
Sandhoff disease, GM2 gangliosidosis II	<i>HEXB</i> 268800	T14F9.3	55	Beta-hexosaminidase beta chain precursor (EC 3.2.1.52)	<i>hex-1</i>	
GM2-gangliosidosis type ab	<i>GM2A</i> 272750	None		Ganglioside GM2 activator precursor		
Krabbe disease	<i>GALC</i> 245200	C29E4.10	31	Galactocerebrosidase precursor (EC 3.2.1.46)		R: None observed
Gaucher disease	<i>GBA</i> 606463	F11E6.1	42	Glucosylceramidase precursor (EC 3.2.1.45)		R: None observed
Variant metachromatic leukodystrophy	<i>PSAP</i> 176801	C28C12.7	21	Proactivator polypeptide precursor, prosaposin	<i>spp-10</i>	M, R: None observed
Variant Gaucher disease	<i>PSAP</i> 176801	C28C12.7	21	Proactivator polypeptide precursor, prosaposin	<i>spp-10</i>	
Variant Tay-Sachs disease (gm2-gangliosidosis)	<i>PSAP</i> 176801	C28C12.7	21	Proactivator polypeptide precursor, prosaposin	<i>spp-10</i>	
Niemann-Pick disease A&B	<i>SMPD1</i> 257200	ZK455.4	55	Sphingomyelin phosphodiesterase precursor (EC 3.1.4.12)	<i>asm-2 d)</i>	R: None observed
		W03G1.7	53		<i>asm-3</i>	M: ND; R: None observed
		B0252.2	52		<i>asm-1 d)</i>	R: None observed
Niemann-Pick disease C1	<i>NPC1</i> 257220	F09G8.4	47	Niemann-Pick C1 protein precursor	<i>ncr-2 (npc-2)</i>	M: hypersensitive to cholesterol deprivation,

			F02E8.6	46		<i>ncr-1 (npc-1)</i>	hyperactive egg-layer, slow development, inappropriate dauer forming; R: Emb M: hypersensitive to cholesterol deprivation, hyperactive egg layer, slow development, inappropriate dauer forming; R: None observed
Fabry's disease, sphingolipidosis	<i>GLA</i> 301500	R07B7.11		54	Alpha-galactosidase A precursor (EC 3.2.1.22)	<i>gana-1 d)</i>	R: Reduced enzyme activity
Schindler disease	<i>NAGA</i> 104170	R07B7.11		53	Alpha-N-acetylgalactosaminidase precursor (EC 3.2.1.49)	<i>gana-1 d)</i>	R: Reduced enzyme activity
<b>Mucopolysaccharidoses</b>							
Mucopolysaccharidosis type I, Hurler/Scheie syndrome	<i>IDUA</i> 252800	None			Alpha-L-iduronidase precursor (EC 3.2.1.76)		
Mucopolysaccharidosis	<i>IDS</i> 309900	None			Iduronate 2-sulfatase precursor (EC		

s type II, Hunter syndrome				35	3.1.6.13)		
Mucopolysaccharidosis type IIIA, Sanfilippo disease IIIA	<i>SGSH</i>	252900	F26H9.1	35	N-sulphoglucosamine sulphohydrolase precursor (EC 3.10.1.1)	<i>chis-1</i>	M: ND, R: None observed
Mucopolysaccharidosis type IIIB, Sanfilippo disease IIIB	<i>NAGL</i>						
Mucopolysaccharidosis type IIIB, Sanfilippo disease IIIB	<i>U</i>	252920	K09E4.4	42	Alpha-N-acetylglucosaminidase precursor (EC 3.2.1.50)		R: None observed
Mucopolysaccharidosis type IIIC, Sanfilippo disease IIIC	<i>HGSN</i>						
Mucopolysaccharidosis type IIIC, Sanfilippo disease IIIC	<i>AT</i>	252930	None		Heparan-alpha-glucosaminide N-acetyltransferase (E.C. 2.3.1.78)		
Mucopolysaccharidosis type IIID, Sanfilippo disease IIID	<i>GNS</i>	252940	K09C4.8	40	N-acetylglucosamine-6-sulfatase precursor (EC 3.1.6.14)	<i>sul-1</i>	M, R: None observed
Mucopolysaccharidosis type IVB, Morquio syndrome B	<i>GLB1</i>	230500	T19B10.3	41	Beta-galactosidase precursor (EC 3.2.1.23)		R: None observed
Mucopolysaccharidosis type IVA, Morquio syndrome A	<i>GALN</i>						
Mucopolysaccharidosis type IVA, Morquio syndrome A	<i>S</i>	253000	D1014.1	41	N-acetylgalactosamine-6-sulfatase precursor (EC 3.1.6.4)	<i>sul-2</i>	M, R: None observed
Metachromatic leucodystrophy	<i>ARSA</i>	250100	D1014.1	40	Arylsulfatase A precursor (EC 3.1.6.8)	<i>sul-2</i>	
Mucopolysaccharidosis type VI, Maroteaux-Lamy	<i>ARSB</i>	253200	C54D2.4	33	Arylsulfatase B precursor (EC 3.1.6.12)	<i>sul-3 d</i>	R: None observed
Mucopolysaccharidosis	<i>GUSB</i>	253220	Y105E8B.9	39	Beta-glucuronidase precursor (EC		R: None

s type VII, Sly syndrome					3.2.1.31)		observed
Hyaluronidase deficiency (Mucopolysaccharidosis type IX)	<i>HYAL1</i>	601492	T22C8.2	31	Hyaluronidase-1 precursor (EC 3.2.1.35)		R: None observed
<b>Lysosomal transporter defects</b>							
Nephropathic cystinosis	<i>CTNS</i>	606272	C41C4.7	48	Cystinosin	<i>ctns-1</i>	M: ND, R: None observed
Infantile sialic acid storage disorder (ISSD) and Salla disease	<i>SLC17A5</i>	604322	C38C10.2	60	Sodium/sialic acid cotransporter, sialin		R: None observed
<b>Lysosomal trafficking defects</b>							
Mucopolysaccharidosis type IV (Hermansky-Pudlak syndrome)	<i>MCO1</i>	605248	R13A5.1	55	Mucopolysaccharidosis type IV Biogenesis of lysosome-related organelles complex 3 component	<i>cup-5</i>	M: maternal-effect embryonic lethal; R: None observed
	<i>HPS1</i>	604982	None				
	<i>AP3B1</i>						
	<i>(HPS2)</i>	603401	R11A5.1	56	AP-3 complex subunit beta-1 Biogenesis of lysosome-related organelles complex 2 component	<i>apb-3 (apt-6)</i>	M: ND; R: Emb, Let, Lva, Dpy, fat content reduced
	<i>HPS3</i>	606118	None				

	<i>HPS4</i>	606682	None		Biogenesis of lysosome-related organelles complex 3 component		
	<i>HPS5</i>	607521	W09G3.6	27	Biogenesis of lysosome-related organelles complex 2 component		R: fat content reduced
	<i>HPS6</i>	607522	None		Biogenesis of lysosome-related organelles complex 2 component		
	<i>DTNB</i> <i>P1</i> ( <i>HPS7</i> )	607145	None		Dystrobrevin-binding protein 1 (Dysbindin), BLOC1 subunit		
	<i>BLOC</i> <i>IS3</i> ( <i>HPS8</i> )	609762	None		Biogenesis of lysosome-related organelles complex-1 subunit 3		
	<i>VPS33</i> <i>A</i>	610034	B0303.9	25	Vacuolar protein sorting 33A	<i>slp-1</i>	M: ND, R: None observed
Mucopolipidosis, types II and IIIA	<i>GNPT</i> <i>AB</i>	607840	None		N-acetylglucosamine-1-phosphotransferase subunits alpha/beta precursor (EC 2.7.8.17)		
Mucopolipidosis, type IIIC	<i>GNPT</i> <i>G</i>	607838	ZK1307.8	38	N-acetylglucosamine-1-phosphotransferase subunit gamma precursor (EC 2.7.8.17)	?	M: ND, R: None observed
<b>Others</b>							
Glycogen storage disease type II, Pompe disease	<i>GAA</i>	232300	D2096.3	37	Lysosomal alpha-glucosidase precursor (EC 3.2.1.20)	?	M, R: ND
Chediak-Higashi syndrome	<i>LYST</i> ( <i>CHSI</i> )	214500	VT23B5.2	32	Lysosomal-trafficking regulator	?	M: ND, R: None observed

			F10F2.1	34			R: response to contact abnormal
Wolman disease / cholesteryl ester storage disease	<i>LIPA</i> 278000	R11G11.14		59	Lysosomal acid lipase/cholesteryl ester hydrolase precursor (EC 3.1.1.13)		R: Him, fat content reduced
Glycogen storage disease type Iib, Danon disease	<i>LAMP</i> 2 300257	C03B1.12		25	Lysosome-associated membrane glycoprotein 2 precursor	<i>Imp-1</i>	M: gut lighter, one type of intestinal granule missing; R:Clr
Sialidosis	<i>NEU1</i> 256550	None			Sialidase-1 precursor (EC 3.2.1.18)		
Multiple sulfatase deficiency	<i>SUMF</i> 1 272200	None			Sulfatase-modifying factor 1 precursor		
Lipoid proteinosis of Urbach and Wiethe	<i>ECM1</i> 602201	None			Extracellular matrix protein 1 precursor		
Dyggve-Melchior-Clausen dysplasia/Smith-McCort dysplasia	<i>DYM</i> 607461	C47D12.2		96	Dymeclin		R: Emb, Let, Muv
Cathepsin E deficiency	<i>CTSE</i> 116890	R12H7.2b)		59	Cathepsin E precursor (EC 3.4.23.34)	<i>asp-4 d)</i>	R: Ced

a) Online Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/Omim/searchomim.html>)

b) Due to high similarity between cathepsin homologues, the highest unidirectional man-worm hit is shown

c) ? Deletion mutant, gene name unknown. Old gene designations are shown between parentheses

d) Gene name assigned, but no mutant available

e) M: Mutant, R: RNAi, ND: not determined Phenotype abbreviations: Age: life span abnormal; Ced: cell death abnormal; Clr: clear; Dpy: dumpy; Emb: embryonal lethal; Him: high incidence of males; Let: lethal; Lva: larval arrest; Lvl: larval lethal; Muv: multi-vulva

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Table 2. *C. elegans* Homologues to Human Genes associated with Lysosomal Function involved in Disorders without known Lysosomal Storage

Human disorders without lysosomal storage caused by lysosomal protein defects	Human Gene	MIM Number a)	<i>C. elegans</i> Homologue	% Similarity to Human Gene	Function/Name	<i>C. elegans</i> Mutant b)	RNAi and mutant phenotypes c)
Renal tubular acidosis with deafness	ATP6V1B2	606939	F20B6.2	92	ATPase, H <sup>+</sup> transporting, lysosomal 56/58kDa V1 subunit B1	<i>vha-12</i>	M: ND; R: Ste Emb Let Adl Lvl Lva Prl Locomotion abnormal
			Y110A7A.12	87		<i>spe-5?</i>	M: Defective spermatogenesis; R: Emb Let Lvl Lva Adl Prl Locomotion abnormal Maternal sterile
Renal tubular acidosis with deafness	ATP6V1B1	192132	F20B6.2	92	ATPase, H <sup>+</sup> transporting, lysosomal 56/58kDa V1 subunit B2	<i>vha-12</i>	
			Y110A7A.12	87		<i>spe-5?</i>	
Renal tubular acidosis, type I	ATP6V0A4	605239	F35H10.4	61	Vacuolar H <sup>+</sup> -ATPase V0 sector, subunit a	<i>vha-5</i>	M: homozygous lethal; R: Emb Let Lvl Lva Gro Prl Pvl Clr Bmd Sck Locomotion abnormal

Pyknodysostosis	CTSK	601105	T03E6.7 T10H4.12	60	Cysteine proteinase Cathepsin K	<i>cpl-1</i>	M: ND; R: Gro Emb Let Locomotion abnormal
Haim-Munk syndrome	CTSC	602365	d)	52	Cysteine proteinase Cathepsin C	<i>cpr-3</i>	M: ND; R: Emb
Papillon-Lefevre syndrome	CTSC	602365					
Juvenile periodontitis	CTSC	602365					
Myeloperoxidase deficiency	MPO	606989	ZK994.3 d)	55	Peroxidase catalyzing hypochlorous acid production	<i>pxn-1</i>	M:ND; R: None observed
Infantile malignant autosomal recessive osteopetrosis	TCIRG1 (OC116, TIRC7)	604592	ZK637.8 d)	57	ATPase, H <sup>+</sup> transporting, lysosomal, V0 subunit A3	<i>unc-32</i>	M: severe coiler; R: Emb Let Gro Sck Locomotion abnormal Maternal sterile Pvl
Infantile malignant autosomal recessive osteopetrosis	CLCN7	602727	R07B7.1	91	Chloride channel 7	<i>clh-6</i>	M:ND; R: None observed
Autosomal dominant osteopetrosis	CLCN7	602727	R07B7.1	91		<i>clh-6</i>	
Infantile malignant autosomal recessive osteopetrosis	OSTM1	607649	F42A8.3	76	Osteopetrosis-associated transmembrane protein 1	?	M:ND; R: None observed
Lowe Syndrome	OCRL	309000	C16C2.3 VF11C1L	86	bisphosphate-5-phosphatase phosphatidylinositol 4-phosphate	<i>ocrl-1 e)</i>	R: None observed M: Ste, Emb, enlarged
Corneal fleck dystrophy	PIP5K3	609414	.1	66	5-kinase	<i>ppk-3</i>	lysosomes; R: None observed
Chorea acanthocytosis	VPS13A	605978	T08G11.1	95	Vacuolar protein sorting 13A		R: None observed
Arthrogryposis, renal dysfunction and	VPS33B	608552	C56C10.1	46	Vacuolar protein sorting 33B		R: None observed

cholestasis

- a) Online Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/Omim/searchomim.html>)
- b) ? Deletion mutant, gene name unknown
- c) M: Mutant, R: RNAi, ND: not determined; Phenotype abbreviations: Adl: Adult lethal; Bmd: organism morphology abnormal; Clr: clear; Emb: embryonal lethal; Gro: slow growth; Let: lethal; Lva: larval arrest; Lvl: larval lethal; Prl: paralyzed; Sck: sick; Ste: sterile
- d) Multiple hits with human protein. No reciprocal best hit due to higher similarity of worm protein to a different human protein. One-directional hit with highest similarity shown
- e) Gene name assigned, but no mutant available

**Table 3. Human genes encoding lysosomal proteins or proteins involved in lysosomal function unassociated with disease**

Human Gene	Protein	MIM Number	C. elegans Homologue	% Similarity to Human Gene	Mutant / CGC Name a)	RNAi and mutant phenotypes b)	KO or spontaneous mouse mutants
<i>ABCA2</i>	ATP binding cassette transporter 2	600047	Y39D8C.1	53	<i>abt-4</i>	M, R: None observed	
<i>ABCA3</i>	ATP binding cassette transporter 3	601615	Y39D8C.1	54	<i>abt-4</i>	M, R: None observed	
<i>ABCA5</i>	ATP binding cassette transporter 5		Y39D8C.1	45	<i>abt-4</i>	M, R: None observed	
<i>ABCB9</i>	ATP binding cassette transporter B9	605453	W04C9.1	41	<i>haf-4</i>	M: Glo; R: Emb Gro Let	
			F43E2.4	40	<i>haf-2</i>	M, R: None observed	
<i>ACP2</i>	Lysosomal acid phosphatase 2	171650	T13B5.3	33		R: None observed	<i>nax, acp2 null</i>
			B0361.7	32		R: None observed	
			F52E1.8	32		R: None observed	
<i>ACP5</i>	Acid phosphatase 5	171640	F02E9.7	36		R: None observed	
<i>ACPT</i>	Acid phosphatase, testicular	606362	B0361.7	34		R: None observed	

			F52E1.8	32		R: None observed	
			T21B6.2	32		R: None observed	
<i>ACPP</i>	Acid phosphatase, prostate	171790	T13B5.3	32		R: None observed	
			R13H4.3	50		R: None observed	
<i>AP3B2</i>	AP-3 adaptor complex, subunit Beta 2	602166	R11A5.1	69	<i>apb-3</i>	M: ND; R: Emb, Let, Lva, Dpy, fat content reduced	
<i>AP3D1</i>	AP-3 adaptor complex, subunit Delta 1	607246	W09G10.4 B	65	<i>apd-3 (apt-5) f)</i>	R: Emb Let Lva Dpy	<i>mocha</i>
<i>AP3M1</i>	AP-3 adaptor complex, subunit Mu1	610366	F53H8.1	80	<i>apm-3 (apt-7) f)</i>	M: Glo; R: Emb Let Lva Dpy fat content reduced	
<i>AP3M2</i>	AP-3 adaptor complex, subunit Mu2		F53H8.1	79	<i>apm-3 (apt-7) f)</i>	M: Glo; R: Emb Let Lva Dpy fat content reduced	
<i>AP3S1</i>	AP-3 adaptor complex, subunit Sigma 1	601507	Y48G8AL. 14	86	<i>aps-3 (apt-8) f)</i>	R: Emb Let Lva Dpy maternal sterile	
<i>AP3S2</i>	AP-3 adaptor complex, subunit Sigma 2	602416	Y48G8AL. 14	88	<i>aps-3 (apt-8) f)</i>	R: Emb Let Lva Dpy maternal sterile	
<i>ARL8B</i>	ADP-ribosylation factor-like 8B		Y57G11C. 13	95	<i>arl-8</i>	R: Emb Let	
<i>ARSD</i>	arylsulfatase D N-acylsphingosine amidohydrolase (acid ceramidase)- like	300002	Arylsulfata se family c)	57			
<i>ASAHL</i>	ATPase, H+	607469	Y55D5A.3	29		R: None observed	
<i>ATP6AP1</i>		<u>300197</u>	Y55H10A.	30	<i>vha-19</i>	M: Let or Ste; R: Ste Sck	

	transporting, lysosomal accessory protein 1 ATPase, H+ transporting, lysosomal accessory		1			
<i>ATP6AP2</i>	protein 2 ATPase, H+ transporting, lysosomal accessory	<u>300556</u>	R03E1.2	23		R: Lva Emb Let Unc Lvl
<i>ATP6V0A1</i>	lysosomal V0 subunit a isoform 1 ATPase, H+ transporting,	<u>192130</u>	ZK637.8f	57	<i>unc-32</i>	M: Unc; R: Ste Pvl Sck Emb Let Unc Gro
<i>ATP6V0A2</i>	lysosomal V0 subunit a isoform 2 ATPase, H+ transporting, lysosomal 21kDa, V0		ZK637.8	47	<i>unc-32</i>	M: Unc; R: Ste Pvl Sck Emb Let Unc Gro
<i>ATP6V0B</i>	subunit b ATPase, H+ transporting,	<u>603717</u>	T01H3.1	64	<i>vha-4</i>	R: Stp Emb Unc Lvl Clr
<i>ATP6V0C</i>	lysosomal 16kDa, V0 subunit c ATPase, H+ transporting,	<u>108745</u>	R10E11.2	67	<i>vha-2</i>	M: ND; R: Ste Emb Let,
<i>ATP6V0D1</i>	lysosomal 38kDa, V0 subunit d1 ATPase, H+	<u>607028</u>	C30F8.2	75	<i>vha-16 f)</i>	R: Let Lvl Lva Emb Maternal sterile
<i>ATP6V0D2</i>	transporting, lysosomal 38kDa, V0		C30F8.2	81	<i>vha-16 f)</i>	R: Let Lvl Lva Emb Maternal sterile

	subunit d2 ATPase, H+ transporting, <i>ATP6V0E</i> lysosomal 9kDa, V0					
<i>1</i>	subunit e1 ATPase, H+ transporting, V0	<u>603931</u>	F49C12.13	71	<i>vha-17</i>	M: ND; R: Ste Sck Emb Lvl Lva
<i>2</i>	subunit e2 ATPase, H+ transporting, lysosomal 70kDa, V1		F49C12.13	75	<i>vha-17</i>	M: ND; R: Ste Sck Emb Lvl Lva
<i>ATP6V1A</i>	subunit A ATPase, H+ transporting, <i>ATP6V1</i> lysosomal 42kDa, V1	<u>607027</u>	Y49A3A.2	82	<i>vha-13 f)</i>	R: Emb Let Gro Lva Prl maternal sterile
<i>C1</i>	subunit C1 ATPase, H+ transporting, <i>ATP6V1</i> lysosomal 42kDa, V1	<u>603097</u>	Y38F2AL. 3A	56	<i>vha-11 f)</i>	R: Ste Emb Let Gro small Sck Lvl Lva Stp maternal sterile decreased broodsize
<i>C2</i>	subunit C2 ATPase, H+ transporting, <i>ATP6V1</i> lysosomal 34kDa, V1		Y38F2AL. 3A	50	<i>vha-11 f)</i>	R: Ste Emb Let Gro small Sck Lvl Lva Stp maternal sterile decreased broodsize
<i>D</i>	subunit D ATPase, H+ transporting, <i>ATP6V1E</i> lysosomal 31kDa, V1		F55H2.2	68	<i>vha-14 f)</i>	R: Emb Let Lva
<i>1</i>	subunit E1 ATPase, H+	<u>108746</u>	C17H12.14	58	<i>vha-8 (pes-6)</i>	M: ND; R: Ste Emb Let Gro Lva decreased broodsize
			C17H12.14	57	<i>vha-8 (pes-6)</i>	M: ND; R: Ste Emb Let Gro Lva

2	transporting, lysosomal 31kDa, V1 subunit E2 ATPase, H+ transporting, lysosomal 14kDa, V1					decreased broodsize
<i>ATP6VIF</i>	subunit F ATPase, H+ transporting, lysosomal 13kDa, V1	<u>607160</u>	ZK970.4	70	<i>vha-9 f)</i>	R: Emb Let Lvl Unc Lva maternal sterile
<i>ATP6V1 G1</i>	subunit G1 ATPase, H+ transporting, lysosomal 13kDa, V1	<u>607296</u>	F46F11.5	53	<i>vha-10 f)</i>	R: Emb Lvl Ste Etv
<i>ATP6V1 G2</i>	subunit G2 ATPase, H+ transporting, lysosomal 13kDa, V1	<u>606853</u>	F46F11.5	35	<i>vha-10 f)</i>	R: Emb Lvl Ste Etv
<i>ATP6V1 G3</i>	subunit G3 ATPase, H+ transporting, lysosomal 50/57 kDa, V1 subunit H		F46F11.5	42	<i>vha-10 f)</i>	R: Emb Lvl Ste Etv
<i>ATP6V1 H</i>	Biogenesis of lysosome-related organelles complex 1 (BLOC1) subunit 1	<u>608861</u>	T14F9.1	45	<i>vha-15 f)</i>	R: Emb Let Lva Prl Lvl Bli
<i>BLOC1S 1</i>	Biogenesis of lysosome-related organelles complex 1	601444	T20G5.10	67		R: None observed
<i>BLOC1S 2</i>	lysosome-related organelles complex 1	609768	Y73B6BL. 30	62		R: None observed

	(BLOC1) subunit 2						
	Biogenesis of lysosome-related organelles complex 1 (BLOC1) subunit 3	609672	None d)				
<i>BLOC1S3</i>							
<i>CD222</i>	IGF2R	147280	F23D12.2	34		R: None observed	
	Lysosomal membrane glycoprotein						
<i>CD63</i>	CD63/GP53	155740	T23D8.2	34	<i>tsp-7</i>	R: None observed	
			T14G10.6	27	<i>tsp-12</i>	M, R: None observed	
			Lectin family c)				
<i>CD68</i>	CD68	153634					
						M: Let, Ste; R: mild endocytosis defect (compared to cup-5)	<i>clcn3</i>
<i>CLCN3</i>	Chloride channel	600580	C07H4.2	45	<i>clh-5</i>		
<i>CLCN6</i>	Chloride channel cappuccino, homolog of mouse, BLOC1 subunit	602726	R07B7.1	60	<i>clh-6</i>	M: ND; R: None observed	<i>clcn6</i>
<i>CNO</i>		605695	None Serine carboxypeptidase, vitellogenic-like				<i>cappuccino</i>
<i>CPVL</i>		609780	family c)	54			
<i>CTBS</i>	Chitobiase	600873	T01C4.1	25		R: None observed	
<i>CTSB</i>	Cathepsin B	116810	F57F5.1 e)	52	?	M: ND; R: Emb Unc Lva	<i>ctsb</i>
			W07B8.5	48	<i>cpr-5 f)</i>	R: None observed	
			C52E4.1	51	<i>cpr-1 (gcp-1)</i>	M: ND; R: None observed	
			F44C4.3	36	<i>cpr-4</i>	M: ND; R: None observed	
			C25B8.3	49	<i>cpr-6</i>	M, R: None observed	

			W07B8.4	49		R: None observed	
			W07B8.1	43		R: None observed	
			F36D3.9	45	<i>cpr-2 f)</i>	R: None observed	
			T10H4.12	45	<i>cpr-3</i>	M: ND; R: Emb Let	
			F32H5.1	40		R: Gro	
			Y65B4A.2	42		R: None observed	
<i>CTSF</i>	Cathepsin F	603539	F41E6.6	49	<i>tag-196</i>	M: mild Unc Egl; R: None observed	<i>ctsf</i>
			R09F10.1	35		R: None observed	
			R07E3.1	35	?	M: ND; R: None observed	
<i>CTSH</i>	Cathepsin H	116820	K02E7.10	37		R: None observed	
			C50F4.3	30	<i>tag-329</i>	M: Gro Lvl decreased broodsize; R: None observed	
			Y113G7B.15	33		R: None observed	
<i>CTSL</i>	Cathepsin L	116880	T03E6.7	49	<i>cpl-1</i>	M: ND; R: Emb Let Gro Unc	<i>ctsl</i>
			Y51A2D.8	32		R: None observed	
			Y71H2AR.2	38		R: None observed	
<i>CTSL2</i>	Cathepsin L2	603308	Y40H7A.1				
<i>CTSS</i>	Cathepsin S	116845	0	33	?	M: ND; R: None observed	
			F15D4.4	30		R: None observed	
						M: ND; R: Emb Let Lva Bmd Mlt maternal sterile	
<i>CTSZ</i>	Cathepsin Z	603169	F32B5.8	55	<i>cpz-1</i>	M: ND, R: None observed	
			M04G12.2	50	<i>cpz-2</i>		
<i>DNASE2</i>	Deoxyribonuclease II, lysosomal	126350	F09G8.2	34	<i>tag-198</i>	M, R: None observed	
	ectonucleoside		C33H5.14				
<i>ENTPD4</i>	triphosphate	607577	c)	32	<i>ntp-1 f)</i>	R: None observed	

	diphosphorylase 4					
<i>GGH</i>	gamma-glutamyl hydrolase major	601509	None			
<i>HLA-DMA</i>	histocompatibility complex, class II, DM alpha major	142855	None			
<i>HLA-DMB</i>	histocompatibility complex, class II, DM beta	142856	None			
<i>HYAL2</i>	Hyaluronidase 2	603551	T22C8.2	31		R: None observed
<i>IFI30</i>	Interferon-inducible protein 30	604664	F37H8.5 C02D5.2	51 50		R: None observed R: None observed
<i>LAMP1</i>	Lysosomal-associated membrane protein 1	153330	C05D9.2	36	<i>lmp-2 f)</i>	R: None observed
<i>LAMP3</i>	Lysosomal-associated membrane protein 3	605883	Lectin family c)			
<i>LAPTM4A</i>	lysosomal associated protein transmembrane 4A		C05E11.3 F23D12.1	46 50		R: None observed R: None observed
<i>LAPTM4B</i>	lysosomal associated protein transmembrane 4B		C05E11.3	45		R: None observed
<i>LAPTM5</i>	Lys.-assoc. multispanning membrane protein-5	601476	C05E11.3	28		R: None observed

<i>LGMN/P</i>	legumain; hydrolysis of asparaginyl bonds	602620	T28H10.3	42		R: Emb Let
<i>RSC1</i>						M: Lvl Mlt; R: Gro Prl Mlt Unc Lvl Bmd
<i>LRP2</i>	Glycoprotein 330 lysophospholipase 3 (lysosomal phospholipase A2)	600073	F29D11.1	30	<i>lrp-1</i>	Dpy Lva Rup Mig
<i>LYPLA3</i>	phospholipase A2) muted, homolog of mouse, BLOC1	609362	M05B5.4	46		R: fat content reduced
<i>MUTED</i>	subunit N-acetylglucosamine-1-phosphodiester alpha-N-	607289	None			<i>muted</i>
<i>NAGPA</i>	acetylglucosaminidase Oxysterol binding	607985	EGF-like domain family c) Y47D3A.1			
<i>OSBP</i>	protein progesterin and adipoQ receptor family	167040	7	64	<i>obr-1 f)</i>	R: Emb Let
<i>PAQR8</i>	member VIII prenylcysteine	607780	K11C4.2	26		R: None observed
<i>PCYOX1</i>	oxidase 1 pallidin, BLOC-1		None			
<i>PLDN</i>	subunit Palmitoyl-protein thioesterase 2	604310	None			
<i>PPT2</i>	precursor prolylcarboxypeptidase	603298	F44C4.5	51	<i>ppt-1</i>	M: delayed egg-laying , abnormal mitochondria; R: None observed
<i>PRCP</i>	e peroxiredoxin 6;	176785	ZK112.1 Y38C1AA.	41	<i>pcp-1 f)</i>	R: None observed
<i>PRDX6</i>	Involved in redox	602316	11	50	<i>prdx-6 f)</i>	R: ND

	regulation of the cell protease, serine 16						
<i>PRSSI6</i>	(thymus)	607169	K12H4.7A	48		R: None observed	
			C26B9.5	44		R: None observed	
			F19C7.2	45		R: None observed	
			F19C7.4	43		R: None observed	
			F23B2.11	46	<i>pcp-3 f)</i>	R: None observed	
			F23B2.12	45	<i>pcp-2</i>	M: ND, R: None observed	
<i>RAB7A</i>	Ras oncogene family member RAB7A	602298	W03C9.3	86	<i>rab-7</i>	M: ND; R: Emb Let Bmd Dpy Prl Ced fat content reduced	
	Lysosomal integral membrane protein II (CD36L2/LGP85/LIM						
<i>SCARB2</i>	P II)	602257	Y49E10.20	28	?	M: ND; R: Pvl maternal sterile	<i>scarb2</i>
			Y76A2B.6	28	?	M: ND; R: None observed	
	solute carrier family 30 member 2, Zinc						
<i>SLC30A2</i>	transporter	609617	T18D3.3	69	?	M: ND, R: None observed	
<i>SLC36A1</i>	solute carrier family 36 member 1	606561	T27A1.5	34		R: RIC	
	snapin, SNARE and						
<i>SNAPAP</i>	BLOC-1 component	607007	C02B10.2	51	?	M: ND; R: Emb M: Egl, slightly bloated, HSN migration defects; R: None observed	
<i>SNX1</i>	sorting nexin 1	601272	C05D9.1	59	<i>snx-1</i>		
<i>STX7</i>	syntaxin 7	603217	F36F2.4	58	<i>syn-13</i>	M: None observed; R: Emb Let	
<i>STX12</i>	syntaxin 12	606892	F36F2.4	58	<i>syn-13</i>	M: None observed; R: Emb Let	
<i>SYBL1/V</i>			Y69A2AR.				
<i>AMP7</i>	synaptobrevin-like 1	300053	6	53		R: None observed	

<i>SYT7</i>	synaptotagmin 7 transmembrane protein 9. Involved in	604146	C08G5.4	62	<i>snt-6</i>	R: None observed
<i>TMEM9</i>	intracellular transport endosomes and lysosomes		R12C12.6	55		R: None observed

a) ? Deletion mutant, gene name unknown

b) Phenotype abbreviations: Bli: blistered; Bmd: organism morphology abnormal, Ced: cell death abnormal; Clr: clear; Dpy: dumpy; Egl: egg laying defect; Emb: embryonal lethal; Etv: embryonic terminal arrest variable emb; Glo: gut fluorescence loss; Gro: slow growth;

Lva: larval arrest; Let: lethal; Lvl: larval lethal; Mig: cell migration abnormal; Mlt: molting defect; Prl: paralyzed; Pvl: protruding vulva; Ric: aldicarb resistant; Rup: exploded through vulva; Sck: sick; Ste: sterile; Stp: sterile progeny; Unc: uncoordinated

c) BLAST hit > 1 e-5, but no reciprocal BLAST hits; when given: similarity with highest hit

d) None: Blast hit < 1 e-5

e) Due to similarity of cathepsin protein sequences, different homologous pairs are shown

f) Gene name assigned, but no mutant available

Table 4. *C. elegans* phenotypes associated with changes in lysosomal function

Mutant or RNAi phenotype	Primary gene Associated with Lysosomal Phenotype	<i>C. elegans</i>		Human Homologue	% Similarity to Human gene	Function of (human) protein	MIM Number	References
		Locus Name	Gene Name					
coelomocyte uptake defect, accumulation of refractile cell corpses	<i>cup-5</i>		R13A5.1	<i>MCOLN1</i>	55	Mucolipin-1	605248	[33]
	<i>cup-1, cup-3, cup-6 - cup-11</i>		?	?				[23] PNAS 99: 4355 (2002)
	<i>cup-2</i>		F25D7.1	<i>DERL1</i>	65	Misfolded protein degradation in ER	608813	
	<i>cup-4</i>		C02C2.3	<i>CHRNB1</i>	43	acetylcholine receptor intracellular	100710	
	<i>rme-1</i>		W06H8.1	<i>EHD1</i>	81	sorting	605888	[23]
	<i>rme-6</i>		F49E7.1	<i>GAPVD1</i>	52	Rab5-		[23]

gut granule biosynthesis defect	<i>glo-1</i>	<i>rme-8</i>	F18C12.2	<i>DNAJC13</i>	64	activating protein 6, endocytosis DnaJ (Hsp40) homolog	[23]
		<i>glo-1</i>	R07B1.12	<i>RAB32</i>	66	Ras oncogen family member RAB32	
		<i>glo-3</i>	?	?			
		<i>glo-4</i>	F07C3.4	<i>HERC4</i>	43	hect domain and RLD 4, ubiquitin ligase ATP binding cassette	
		<i>pgp-2 (glo-5)</i>	C34G6.4	<i>ABCB1</i>	66	transporter B1	171050
		<i>haf-4</i>	W04C9.1	<i>ABCB9</i>	41	transporter B9	605453
		<i>apt-6</i>	R11A5.1	<i>AP3B1 (HPS2)</i>	56	AP-3 complex subunit beta-1	603401
		<i>apt-7</i>	F53H8.1	<i>AP3M1</i>	80	AP-3 complex, subunit Mu1	610366
		<i>vps-16</i>	C05D11.2	<i>VPS16</i>	44	Vacuolar protein sorting-associated protein	608550
							Mol Biol Cell 18: 995 (2007)

	<i>vps-41</i>	F32A6.3	<i>VPS41</i>	51	16 homolog Vacuolar protein sorting- associated protein 41 homolog	605485	
loss of one type of intestinal granule	<i>lmp-1</i>	C03B1.12	<i>LAMP1</i>	57	Lysosomal- associated membrane protein	153330	[22]
vacuolated intestinal cells, egg retention,	<i>cad-1</i>	?	?				Genetics 119: 355 (1988) J Biol Chem 275: 26359 (2000)
reduced aspartic protease activity, enzyme processing defect?							
slow development, premature egg laying	<i>ncr-1</i>	F02E8.6	<i>NPC1</i>	46	Niemann-Pick C1 protein precursor	257220	[44]
dauer-constitutive (Daf-c) phenotype	<i>ncr-1; ncr-2</i>	F09G8.4	<i>NPC1</i>	47	Niemann-Pick C1 protein precursor	257220	[44]
DNA in intestinal lumen	<i>nuc-1</i>	C07B5.5	<i>DNASE2B</i>	54	deoxyribonucle		Genetics

not degraded						ase II beta	129: 79 (1991)
major endodeoxyribonuclease reduced >95% condensed chromatin persists after apoptosis							Science 220: 1277 (1983)
acc of enlarged lysosomes, paralysis during 1st larval stage, larval arrest, persistent apoptotic corpses	<i>idi-1</i>		K06H7.9	<i>ID11</i>	56	isopentenylidip hosphate isomerase	604055 Mol Gen Genomics 273: 158 (2005)
coiler; very sluggish, moves poorly; slightly Egl slightly short; defecation defects, abnormal FITC staining; subviable. Abnormal adult males tails and spicules	<i>unc-101</i>	<i>unc-101</i>	K11D2.3	<i>AP1M1</i>	88	AP-1 complex, subunit Mu1	603535 Genes Dev. 8: 60 (1994)