

Maintenance of Membrane Integrity and Permeability Depends on a Patched-Related Protein in *Caenorhabditis elegans*

Myung-Kyu Choi,^{*1,2} Sangwon Son,^{*1} Mingi Hong,^{*3} Min Sung Choi,[†] Jae Young Kwon,[†] and Junho Lee^{*,†4}

^{*}Department of Biological Sciences, Institute of Molecular Biology and Genetics and [†]Department of Biophysics and Chemical Biology, Seoul National University, 08826 Seoul, Korea, and [†]Department of Biological Sciences, Sungkyunkwan University, Suwon, 16419 Gyeonggi-do, Korea

ABSTRACT Membrane integrity is critical for cell survival, defects of which cause pathological symptoms such as metabolic diseases. In this study, we used ethanol sensitivity of the nematode *Caenorhabditis elegans* to identify genetic factors involved in membrane integrity. In *C. elegans*, acute exposure to a high concentration (7% v/v) of ethanol changes membrane permeability, as measured by propidium iodide staining, and causes paralysis. We used the timing of complete paralysis as an indicator for alteration of membrane integrity in our genetic screen, and identified *ptr-6* as a gene that confers ethanol resistance when mutated. PTR-6 is a patched-related protein and contains a sterol sensing domain. Inhibition of two PTR-encoding genes, *ptr-15* and *ptr-23*, and *mboa-1*, encoding an Acyl Co-A: cholesterol acyltransferase homolog, restored ethanol sensitivity of the *ptr-6* mutant, suggesting that these *ptr* genes and *mboa-1* are involved in the maintenance of membrane integrity and permeability. Our results suggest that *C. elegans* can be used as a model system to identify factors involved in metabolic diseases and to screen for therapeutic drugs.

KEYWORDS *Caenorhabditis elegans*; alcohol; Patched-related; membrane integrity

PROPER composition and organization of cellular membranes are important for signaling pathway, cell polarity, and membrane structural stability. Phospholipid composition and thus membrane integrity is often altered in Alzheimer's disease and metabolic diseases such as atherosclerosis and hyperlipidemia (Gillies and Robinson 1988; Muller *et al.* 1990; Engelmann *et al.* 1992). Although changes in membrane integrity have been studied intensively in artificial membranes, isolated tissues, and unicellular organisms

(Harrison and Vickers 1990; Jung and Levin 1999; Kusumi *et al.* 2005), the genetic and molecular mechanisms regulating membrane integrity at the multicellular organismal level are largely unknown.

Under experimental conditions, membrane integrity can be altered by chemical stresses such as alcohols and detergents. Classical studies found that ethanol influences the fluidity of cell membranes (Chin and Goldstein 1977; Johnson *et al.* 1979; Dombek and Ingram 1984). Therefore, we reasoned that genetic screens using ethanol as a reagent to alter membrane integrity could lead to isolation of mutants involved in the regulation of membrane integrity. In previous studies, we established a genetic system to identify genes involved in the ethanol response of *Caenorhabditis elegans* and isolated ethanol-resistant mutants (Kwon *et al.* 2004; Hong *et al.* 2008). Realizing that our previous genetic screens might have uncovered mutants involved in the regulation of membrane integrity, we decided to revisit the previously isolated ethanol-resistant mutations. In this study, we analyze the previously isolated *ys20* mutant, which we identify as a

Copyright © 2016 by the Genetics Society of America
doi: 10.1534/genetics.115.179705

Manuscript received June 22, 2015; accepted for publication February 2, 2016; published Early Online February 5, 2016.

Supplemental material is available online at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.179705/-/DC1.

¹These authors equally contributed to this work.

²Present address: Department of Organismic and Evolutionary Biology, Center for Brain Science, Harvard University, Cambridge, MA 02138.

³Present address: Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY 10029.

⁴Corresponding author: Department of Biological Sciences, Department of Biophysics and Chemical Biology, Institute of Molecular Biology and Genetics, 105-319, Seoul National University, Gwanak-ro 1, 08826 Gwanak-gu, Seoul, Korea.
E-mail: elegans@snu.ac.kr

mutation in the *ptr-6* gene. Through further genetic analysis, we propose that cholesterol metabolism is involved in membrane integrity. Our study suggests that the nematode *C. elegans* can be utilized as a model system to screen for therapeutic treatments for diseases such as atherosclerosis.

Materials and Methods

Strains and culture

C. elegans worms were grown on nematode growth media (NGM) agar plates seeded with OP50 bacteria at 20° as described (Brenner 1974). Mutant strains were obtained from the Caenorhabditis Genetic Center or EMS mutagenesis (Hong *et al.* 2008). Wild type was the N2 Bristol strain. The strains used were as follows: *ptr-6(ys9)*, *ptr-6(ys20)*, *ptr-20(ok2988)*, *ptr-10(ok2106)*, N2;Ex[Pptr-6::GFP, pRF6(*rol-6(su1006)*)], *ptr-6(ys20)*;Ex[Pdpy-7::ptr-6::GFP, pRF6(*rol-6(su1006)*)], *ptr-6(ys20)*;Ex[Pmyo-3::ptr-6::GFP, pRF6(*rol-6(su1006)*)], N2;Ex[mboa-1 (+), pRF6(*rol-6(su1006)*)] *rde-1(ne219)*;kzIS9[*Plin-26::nlp::GFP*, *Plin-26::rde-1*, pRF6(*rol-6(su1006)*)], and *rde-1(ne219)*;kVIS20[*Phlh-1::rde-1*, *Psur-5::nls::GFP*].

DNA constructs

To examine the *ptr-6* expression pattern, 2 kb of the 5' upstream region of the gene was combined with GFP by PCR fusion (Hobert 2002). For tissue-specific rescue experiments, we used the GATEWAY system (Invitrogen). The *ptr-6* genomic DNA (gDNA) was cloned into the pJL1007 vector, which is a middle entry vector containing the multicloning sites and GFP region from pPD95.77. *ptr-6* gDNA was amplified by PCR with *C. elegans* gDNA as the template. Entry vectors for the promoters were obtained from the Promoterome library (Dupuy *et al.* 2004). For the *mboa-1* overexpression experiment, genomic DNA containing 2 kb of the 5' upstream region and the entire coding region of the *mboa-1* gene was amplified by PCR.

The primers used were as follows: *ptr-6*(RNAi)-F AACTGCAGATCATTGCTGTCCAGGGTTT; *ptr-6*(RNAi)-R AAGGTACCAATGGGTCAGAATGGAGCAG; PF-A *Pptr-6* AGTTTGTCTGACAGTTTTCTTA; PF-B *Pptr-6* AGTCGACCTGCAGGCATGCAAGCTCATCTGGAAATTTTAAGA GACTC; PF-C *GFP* AGCTTGCATGCCTGCAGGTCG; PF-D *GFP* AAGGGCCCGTACGGCCGACTA; PF-D* *GFP* GGAAAAAGT TATGTTTGGTATA; *ptr-6* (pJL1007)-F AAG CTT ATG CGA TGC CGG ATT CCA ACT CTT G; *ptr-6* (pJL1007)-R TCT AGA CGA GGA TAC ATT TAC CGG CAA ATT AAT TGC; *mboa-1*-F (O/E) GCCGAACTGTACAGGGTTTCT; and *mboa-1*-R(O/E) TATCAAGTAACTAATATTTATTTTCTG.

Statistical analysis

In ethanol resistance assays, statistical significance was determined by a log-rank analysis using OASIS (Yang *et al.* 2011). In staining experiments and the freezing–thawing assay, statistical significance was determined using one-way ANOVA and Bonferroni's multiple-comparison post-test.

Behavior assay

To immerse *C. elegans* worms in 1-ml ethanol solutions of defined concentrations, ~20 young worms were washed off agar plates with ethanol dissolved in M9 buffer at known concentrations and placed in clear 55-mm petri dishes. Moving worms were scored over time. We used this ethanol assay to isolate and identify mutation. Another method used was to pick ~20 young adult worms in clear wells containing 200- μ l ethanol solutions of defined concentrations and score for motility over time until all individuals ceased movement. Ethanol sensitivity measured by picking worms was not significantly different from the result by immersing. With all data, we used the picking method. Each experiment was repeated three times independently. For every strain, a control experiment using M9 buffer alone was done to check basal locomotion activity. It was normal until the 20-min time point.

The sodium azide sensitivity test was done similarly to the ethanol test. We put 20 young adult worms in clear dishes containing 25 mM sodium azide with M9. Acute osmotic stress assay was performed using 500 mM NaCl plates as described (Solomon *et al.* 2004).

Freezing and thawing assay

To check the survival ratio after freezing and thawing quantitatively, we used a previously defined liquid freezing solution with defined glycerol concentrations (Brenner 1974). The freezing solution samples were divided into four groups and frozen after 0, 10, 20, and 30 min of incubation at room temperature, respectively. After 1 week in the –80 freezer, we thawed the samples at room temperature. We allocated the solution in three NGM plates and then measured the numbers of total animals and live animals.

Identification of *ptr-6* as a gene that confers ethanol resistance

Ethanol-resistant mutants were previously isolated by EMS mutagenesis (Hong *et al.* 2008). The *ys9* and *ys20* mutations were genetically mapped by SNP mapping (Wicks *et al.* 2001). Both mutants were located in the middle region [II: 23.3II: 1.2 map unit (m.u.)] of chromosome II. By testing F1 heterozygotes from crossing *ys9* and *ys20* homozygotes, we found that these two mutations do not complement each other and that they are alleles of the same gene, previously named *jud-1* (Hong *et al.* 2008). By whole-genome sequencing of the *ys9* and *ys20* mutants and comparison with the wild-type N2 sequence, we obtained a list of single-nucleotide changes in *ys9* and *ys20*. We selected the candidate genes that contained the detected mutations and were located within the SNP mapping region (II: –3.3~II: 1.2 m.u.). Because *ys9* and *ys20* were isolated from independent mutagenesis, background mutations were filtered by subtracting identical nucleotide changes found in both *ys9* and *ys20*. *ptr-6* was the only gene mutated in both *ys9* and *ys20*, with mutations in different

regions of *ptr-6*. Rescue experiments were performed by injecting a wild-type version of *ptr-6* into the *ys20* mutant.

Microinjection and microscopy

Microinjection of DNA into the gonads of adult hermaphrodites was carried out according to standard procedures (Mello *et al.* 1991). Young adult hermaphrodites were picked to a 2% agarose pad using halocarbon oil. After injection into their gonads, worms were quickly recovered with M9 buffer. The pRF4 plasmid, which contains the dominant *rol-6* (*su1006*) gene, was used as an injection marker (100 ng/ μ l), and *Psur-5::GFP* and *Pact-5::GFP* were used as markers for transgenic worms used in behavior assays. Most plasmids were injected at the concentration of 100 ng/ μ l. Plasmid DNAs used for injection were extracted with the QIAGEN plasmid midi kit (catalog no. 12145) or the Axygen midi prep kit (catalog no. AP-MD-P-25). To observe the transgene expression patterns, transgenic animals were mounted on 5% agar pads with 2.5 mM levamisole, and fluorescence was observed using an Axioplan 2 microscope or LSM700 confocal microscope (Zeiss).

Feeding RNA interference method

In most cases, clones in the *C. elegans* RNA interference (RNAi) library from the Ahringer Library (Cambridge, UK) were used. Bacteria containing the RNAi clone targeting the gene of interest were streaked and cultured in LB containing ampicillin. Transcriptional activation was induced by adding 1 mM IPTG. For *ptr-6* that did not have proper RNAi clones in the Ahringer Library, 748 bp of genomic fragment was cloned into pPD129.36 (L4440) with *Pst*I and *Bam*HI. Bleached embryos or L1 larvae were cultured on the RNAi plates until they reached the L4-to-young-adult stages, when they were harvested for analysis of ethanol sensitivity. *ptr-6(ys20)* animals were used for the RNAi experiments. In tissue-specific RNAi experiments, we used NR222 (hypodermis) and NR350 (muscle) strains, which are *rde-1* background and rescued in each specific tissue (Qadota *et al.* 2007).

Chemical staining

For the propidium iodide (PI) assay, worms were rinsed with M9 buffer containing 10% (v/v) PI solution with/without 7% (v/v) ethanol. Images were taken by the Axioplan 2 microscope (Zeiss) with the same exposure time. Fluorescence intensity was analyzed by ImageJ software. For CE-bodipy staining, worms were frozen before staining. After thawing, worms were incubated in CE-bodipy solution. Images were taken with an LSM 700 confocal microscope (Zeiss). For quantification, we analyzed the data from 20 animals for each group.

Data availability

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article.

Results

Acute exposure to a high concentration of ethanol alters membrane permeability and causes paralysis in *C. elegans*

To study the effects of ethanol exposure on biological membranes, we performed PI staining in the nematode *C. elegans*. PI is an intercalating agent and is highly fluorescent when bound to nucleic acids. Since PI is membrane-impermeable, it cannot stain normal, living cells. Only the intestinal lumen was stained when worms were treated with PI in M9 buffer (Figure 1A) because PI dye ingested by the mouth into the intestinal lumen could not penetrate into cells. When ethanol exposure was simultaneously conducted with PI treatment by immersing worms in a 7% (v/v) ethanol solution for 20 min, the inside of the pharyngeal region was stained as well as the intestinal lumen (Figure 1, A and B). Consistent with the changes in membrane permeability induced by ethanol, *C. elegans* worms were paralyzed within 10 min upon 7% (v/v) ethanol exposure (Figure 1C). To exclude the possibility that PI staining in ethanol-treated N2 was due only to lethality, we further conducted the recovery experiments. After being treated with 7% (v/v) ethanol and PI dye for 30 min, paralyzed worms were moved to M9 buffer. We found that all of them were recovered and started moving again within 10 min (Supplemental Material, Figure S1), indicating that the viability of the worms after PI treatment was not affected. The correlation of membrane permeability and animal paralysis suggested that a behavioral assay using 7% (v/v) ethanol treatment could be used to identify factors involved in maintaining membrane integrity in *C. elegans*. Since we had previously isolated ethanol-resistant mutants by random mutagenesis (Hong *et al.* 2008), we decided to re-analyze the uncloned mutants to see if they corresponded to factors involved in the maintenance of membrane integrity.

ys20 ethanol-resistant mutant is defective in membrane integrity

The *ys20* allele had been observed to have the strongest resistance to ethanol treatment among the previously isolated ethanol-resistant mutants (Hong *et al.* 2008). Therefore, we decided to first analyze this mutant further. While *ys20* mutant animals showed normal morphology and motility under the dissection microscope in the absence of ethanol, and their growth and reproduction were also normal, PI did not penetrate into the pharyngeal cells of *ys20* mutant animals even in the presence of ethanol (Figure 1A). *ys20* mutant animals had a low survival rate after freezing and thawing for storage in standard freezing solution containing 15% glycerol, even after extensive outcrossing with wild-type N2 animals (Figure S2) (Hong *et al.* 2008), again suggesting that ethanol resistance is likely to be related to membrane properties such as rigidity and integrity.

Next, we examined whether the *ys20* mutant was resistant to other chemicals (Figure 2, B and C). Sodium azide, which can act as a mitochondria inhibitor, is a well-known anesthetic

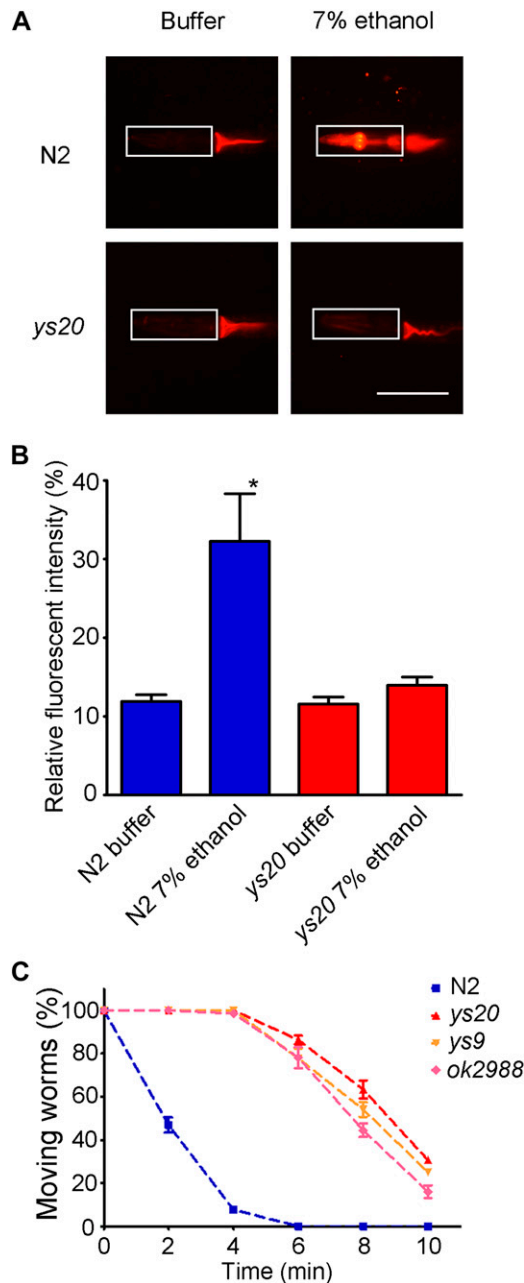


Figure 1 A high concentration of ethanol can affect membrane integrity and induce paralysis. (A) Representative images of a membrane integrity assay using PI upon 7% (v/v) ethanol treatment. Wild-type N2 animals show increased staining inside the pharynx after ethanol treatment. *ys20* mutant animals were not stained in the pharynx. Bars, 150 μ m. (B) Quantitative analysis of PI staining. * $P < 0.01$. (C) The Ethanol response of *ptr-6(ok2988)*, *ys9* and *ys20* mutants to 7% (v/v) ethanol. The *ptr-6(ok2988)* deletion strain showed an ethanol-resistant phenotype similar to that of the *ys9* or *ys20* mutations.

in *C. elegans* (Massie *et al.* 2003). *ys20* animals did not exhibit resistance to sodium azide compared to wild-type animals (Figure 2B). However, *ys20* animals showed resistance to other alcohols such as methanol and isopropanol, at different working concentrations from that of ethanol, and also exhibited resistance to acetone treatment (Figure 2C). These

data indicate that the resistance phenotype of the *ys20* mutation is not specific to ethanol, but broadly applicable to membrane-solubilizing agents. Acetone is not an alcohol, but has a similar property to alcohol in that it can work as a membrane-solubilizing organic solvent (Sikkema *et al.* 1995). Organic solvents can integrate into the plasma membrane and render the membrane permeable (Isken and de Bont 1998). The defect of *ys20* mutants in survival after freezing and thawing may be caused by the impermeability of the membranes of *ys20* mutants for glycerol, which is a main organic solvent for freezing. To test this possibility, we divided the N2 and *ys20* worms in the freezing solution into four groups, incubated at room temperature for 0, 10, 20, and 30 min, respectively, before freezing, and froze them in the -80 freezer. Interestingly, after thawing, *ys20* animals showed an increased survival rate in 20- and 30-min samples compared to 0 min (Figure 2A). These phenotypic analyses suggest that the molecular function of the gene corresponding to the *ys20* mutation is related to regulation of membrane integrity.

***ys20* is an allele of the *jud-1* gene, which corresponds to the *ptr-6* gene**

We mapped *ys20* to a similar region with *ys9*, another previously isolated ethanol-resistant mutant, on chromosome II by SNP mapping. A complementation test using ethanol assay showed that these mutations were alleles of the same gene. After we generated a male from *ys9*, we mated *ys9* males with *ys20* hermaphrodites and tested ethanol sensitivity of F1 male heterozygotes of the genotype *ys9/ys20*. While N2 males and *ys9* heterozygous males of the genotype *ys9/+* showed paralysis within 5 min in 7% ethanol, almost half of the *ys9/ys20* heterozygotes showed motility after 8 min, which was similar to the ethanol sensitivity of *ys9* and *ys20* mutants (Figure S4). *ys9* was previously known to be an allele of the uncloned *jud-1* gene (Hong *et al.* 2008). Using rescue experiments with a candidate gene uncovered by whole-genome sequencing, we found that the previously uncloned *jud-1* gene corresponded to the *ptr-6* gene (*Patched-related family member-6*) (Figure 3, A and D). PTR-6 has 11 transmembrane domains and a sterol-sensing domain (SSD) (Figure 3B) (Zugasti *et al.* 2005) and is homologous to human PTCHD3, which defines one of seven paralogous families of SSD proteins. The *ptr-6 (ys9)* allele has a splicing acceptor mutation in the sixth exon of *ptr-6*, and the *ptr-6 (ys20)* allele a nonsense mutation in the ninth exon of *ptr-6* (Figure 3, A and B). In addition, *ptr-6* RNAi in a wild-type background phenocopied ethanol resistance (Figure S3A). *ok2988*, a *ptr-6* deletion mutant provided by the Caenorhabditis Genetics Center, also showed an ethanol-resistant phenotype similar to the *ys9* and *ys20* mutants (Figure 1C). Although it was reported that *ptr-6* was required for molting together with *ptr-1* and *ptr-10*, we found that knockdown of *ptr-1* and *ptr-10* mutations did not cause any phenotype related to alcohol sensitivity (Figure S5), suggesting that *ptr-6* has a role distinctive from these genes.

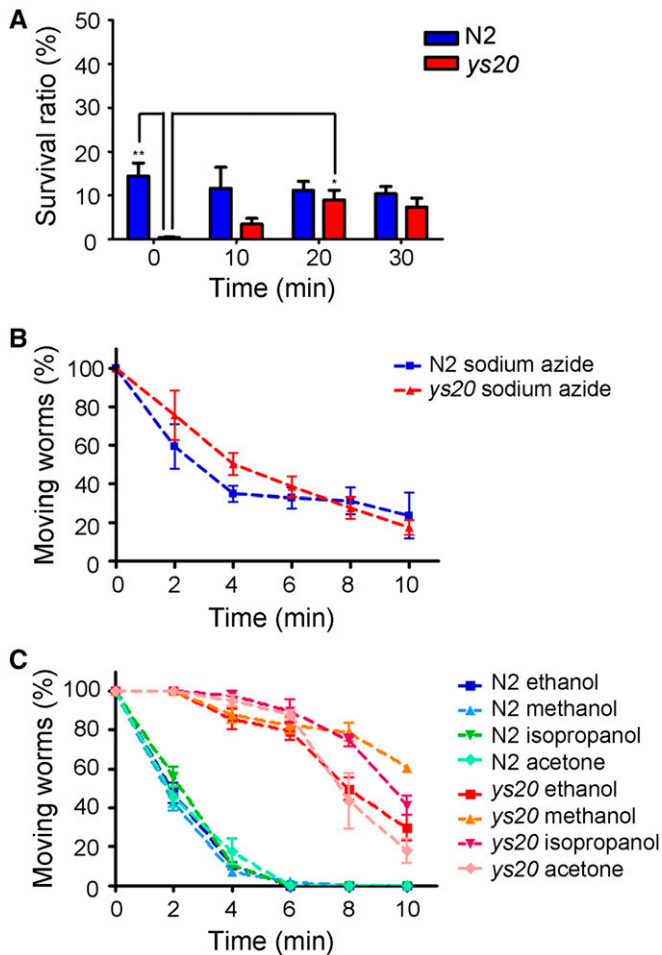


Figure 2 The *ys20* ethanol-resistant mutant shows membrane-related phenotypes. (A) Survival ratio of wild-type and *ys20* mutant animals after freezing and thawing. The x-axis indicates the time of incubation at room temperature before freezing. (B) Time-course sensitivity of N2 and *ys20* mutant animals to sodium azide. (C) Time-course sensitivity of N2 and *ys20* mutant animals to ethanol, methanol, isopropanol, and acetone.

To check the expression pattern of *ptr-6*, a transcriptional fusion of the *ptr-6* promoter and GFP was constructed. *ptr-6* was expressed in the hypodermis, especially strongly in the head region (Figure 3C). To test whether *ptr-6* in the hypodermis played a role for ethanol sensitivity, we made translational fusions of tissue-specific promoters and the *ptr-6*-coding region. *ptr-6* mutants containing a hypodermis-specific rescue construct (*Pdpy-7::ptr-6::GFP*) showed a reduced ethanol resistance phenotype, similar to wild-type worms (Figure 3D). To check whether any extrachromosomal array or injection marker itself could diminish ethanol resistance of *ptr-6*, we tested the muscle-specific expression line (*Pmyo-3::ptr-6::GFP*) as a negative control. Ethanol sensitivity of muscle-specific expression lines was comparable with that of wild-type animals (Figure 3D). We also found that feeding RNAi of *ptr-6* was effective in N2 background (Figure S1A). We wanted to confirm the role of *ptr-6* in the hypodermis using tissue-specific RNAi

strains. Tissue-specific RNAi strains were generated by tissue-specific rescue of *rde-1* in *rde-1* mutant background (Qadota *et al.* 2007). We could observe a *ptr-6* RNAi effect for ethanol sensitivity in hypodermis-specific RNAi strains, but not in muscle-specific RNAi strains (Figure S1, B and C).

Other *ptr* genes are involved in the regulation of membrane integrity

We performed an RNAi screen in search of suppressors of *ptr-6*(*ys20*) mutant phenotypes to gain insight into the molecular and genetic mechanisms of *ptr-6* function in the membrane. Because hedgehog signaling is not conserved in *C. elegans*, we wanted to get possible genetic interactors with *ptr-6*. Based on the fact that PTR proteins are known to contain the sterol-sensing domain (Zugasti *et al.* 2005), we chose 117 sterol, steroid-related genes as candidates to test from the WormBase database (<http://www.wormbase.org>) (Table S1). Among them, *ptr-15* or *ptr-23* RNAi were observed to suppress the *ptr-6* ethanol-resistant phenotype (Figure 4, A and B). Knockdown of *ptr-15* and *ptr-23* in wild-type N2 animals also caused enhancement of ethanol sensitivity, indicating that the endogenous *ptr-15* and *ptr-23* genes can directly influence ethanol sensitivity and may not need to act through the *ptr-6*-mediated pathway to exert their effects (Figure 4, A and B). Like *ptr-6*, *ptr-15* function has not yet been studied. Using a hypodermis-specific RNAi experiment, we found that the site of *ptr-15* action is the hypodermis (Figure 4C). Although *ptr-23* is known to be involved in the osmotic stress response (Rohlfing *et al.* 2011), suppression of *ptr-6* ethanol resistance by *ptr-23* RNAi is not related to the osmotic response because *ptr-6* mutant animals did not show any differences in the osmotic stress response compared to wild-type animals (data not shown).

Among 117 screening candidates, most genes did not affect ethanol sensitivity. It is possible that some family with many paralogous genes in *C. elegans* such as hedgehog-like ligands or lipid-binding proteins can be redundant for ethanol sensitivity and membrane regulation. Interestingly, *nhr-25* can suppress ethanol resistance of *ptr-6*. It suggests that *ptr-6* can affect transcription of a certain gene that is important for membrane regulation.

mboa-1, an acyl-coenzyme A:cholesterol O-acyltransferase homolog, may act through *ptr-6* to influence lipid levels and influence ethanol sensitivity

Using further RNAi screening for *ptr-6* suppressors among sterol, steroid-related genes, we found that knockdown of *mboa-1*, a homolog of acyl-coenzyme A: cholesterol O-acyltransferase (ACAT), an enzyme that forms cholesteryl ester from cholesterol, strongly suppresses the ethanol-resistant phenotype of the *ptr-6* mutation (Figure 5, A and B). ACAT protects the membrane from an excess of free cholesterol by producing cholesteryl ester (CE), which cannot integrate into or interfere with the cell membranes (Cases *et al.* 1998). It suggests that balance between the levels of free cholesterol and cholesteryl ester plays an

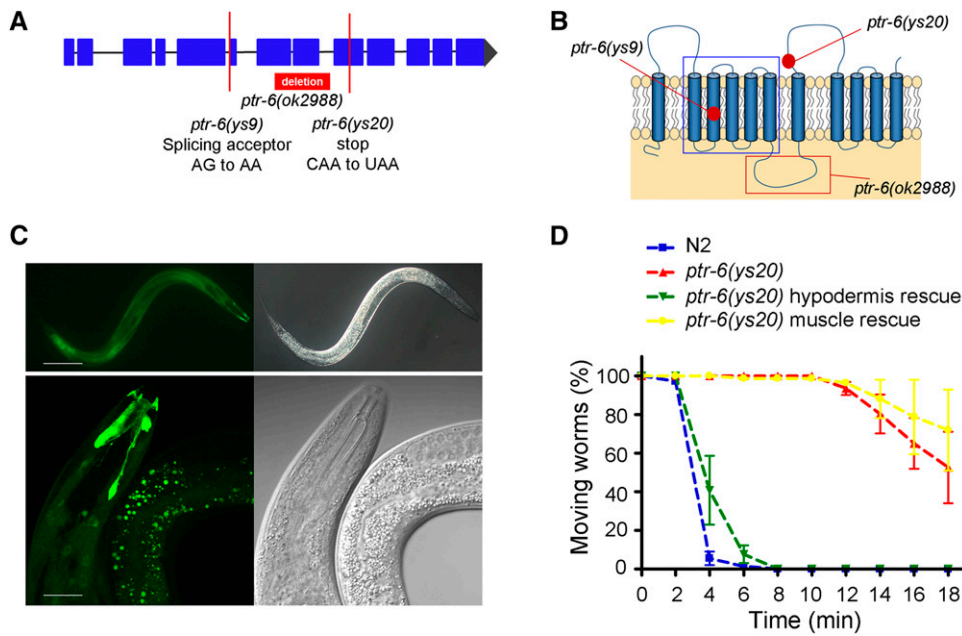


Figure 3 *ptr-6* is expressed in and acts in the hypodermis to regulate membrane integrity. (A) Gene structure of *ptr-6*. The *ys9* and *ys20* mutation sites are indicated as lines. The red box indicates the region deleted in the *ok2988* allele. (B) Predicted protein structure of PTR-6 and the sites of the *ys9* and *ys20* mutations. The red box is the deleted portion of PTR-6 in the *ok2988* allele. The blue box indicates the putative SSD domain. (C) *ptr-6* expression pattern. A transgenic construct with the *ptr-6* promoter fused to GFP is expressed along the hypodermis. Signal intensity was strong in the head region. Bar (upper), 100 μ m; bar (lower), 20 μ m. (D) *ptr-6* tissue-specific rescue experiment. A hypodermis-specific *ptr-6* expression line, *ptr-6(ys20);[Pdpi-7::ptr-6::GFP]*, shows altered ethanol sensitivity similar to wild type. In contrast, a muscle-specific expression line, *ptr-6(ys20);[Pmyo-3::ptr-6::GFP]*, did not show any difference compared to the *ptr-6(ys20)* mutant.

important role in regulating membrane properties. To test this hypothesis, we overexpressed *mboa-1* and checked ethanol sensitivity. *mboa-1* overexpression in wild-type animals caused an ethanol-resistant phenotype (Figure 5C), suggesting that levels of cholesteryl esters may be critical for ethanol sensitivity. When the amounts of cholesteryl esters in wild-type and *ptr-6* mutant animals were analyzed by BODIPY-CE staining, we observed that the amount of cholesteryl esters in the hypodermis was increased in *ptr-6* mutants compared to wild-type N2 animals (Figure 5, D and E). In contrast, the amount of cholesteryl esters in the intestine was slightly decreased in *ptr-6* mutants. In addition to the fact that PTR-6 is potentially a sterol transporter, these data suggest that PTR-6 acts negatively on the amounts of cholesteryl esters stored in the hypodermis. *mboa-1* is known to express in the hypodermal cells (Hunt-Newbury *et al.* 2007). Although we do not rule out the possibility that *mboa-1* in other tissues could play a role in ethanol sensitivity, we speculate that a major site where *mboa-1* and *ptr-6* interact might be the hypodermis because increased BODIPY-CE staining in *ptr-6* is mostly prominent in the hypodermis.

Discussion

In this study, we have shown that PTR-6, a Patched-related protein, and MBOA-1, which may act in sterol homeostasis, are involved in maintaining the membrane integrity using *C. elegans* as a model system. We identified *ptr-6* as a gene involved in membrane integrity by unbiased forward genetic screening using ethanol sensitivity as a read-out of the membrane integrity at the organismic level. Then we utilized a targeted candidate approach for the new players and identified two more *ptr* genes and *mboa-1* as genes involved in membrane integrity.

Patched is a well-known principal component of the Hedgehog pathway. In many organisms, the Hedgehog ligand binds to the Patched receptor in the membrane, releasing Smoothened (smo) from the repressive activity of Patched. The Smoothened signal exerts its effects through the Gli/Ci transcription factor. However, Smoothened is absent from the *C. elegans* genome, leading to the belief that the Hedgehog pathway is not conserved in *C. elegans* (Hausmann *et al.* 2009). Also, the *C. elegans* paralog of Gli/Ci shows a restricted expression pattern in the germ line and seems to be important only for sex determination. Other conserved components of the Hedgehog pathway such as Patched or Hedgehog are not involved in sex determination (Zugasti *et al.* 2005). Even though the key components of the Hedgehog pathway are not conserved in *C. elegans*, the number of Patched-related genes is expanded to 23. It is in contrast to the fact that each genome of mice, *Drosophila*, and humans has only one Patched-related gene. The expansion of Patched-related genes is a feature of Nematoda (Zugasti *et al.* 2005), and it is thought that they have distinctive cellular functions. Some Patched-related genes are partially redundant in molting and cytokinesis, and some have specific functions in glia cell development or response to osmotic stress (Perens and Shaham 2005; Rohlfing *et al.* 2011). We focused on the fact that *ptr* genes contain the sterol-sensing domain. Other unicellular organisms including bacteria have proteins structurally similar to Patched, even though the hedgehog pathway is not conserved in them either. The RND transporter, which is an ancient form of Patched family proteins, contains a sterol-sensing domain and has a role in sterol transport (Ioannou 2001; Hausmann *et al.* 2009). The human patched receptor is also reported to be involved in cholesterol transport (Bidet *et al.* 2011). These previous studies and our data suggest

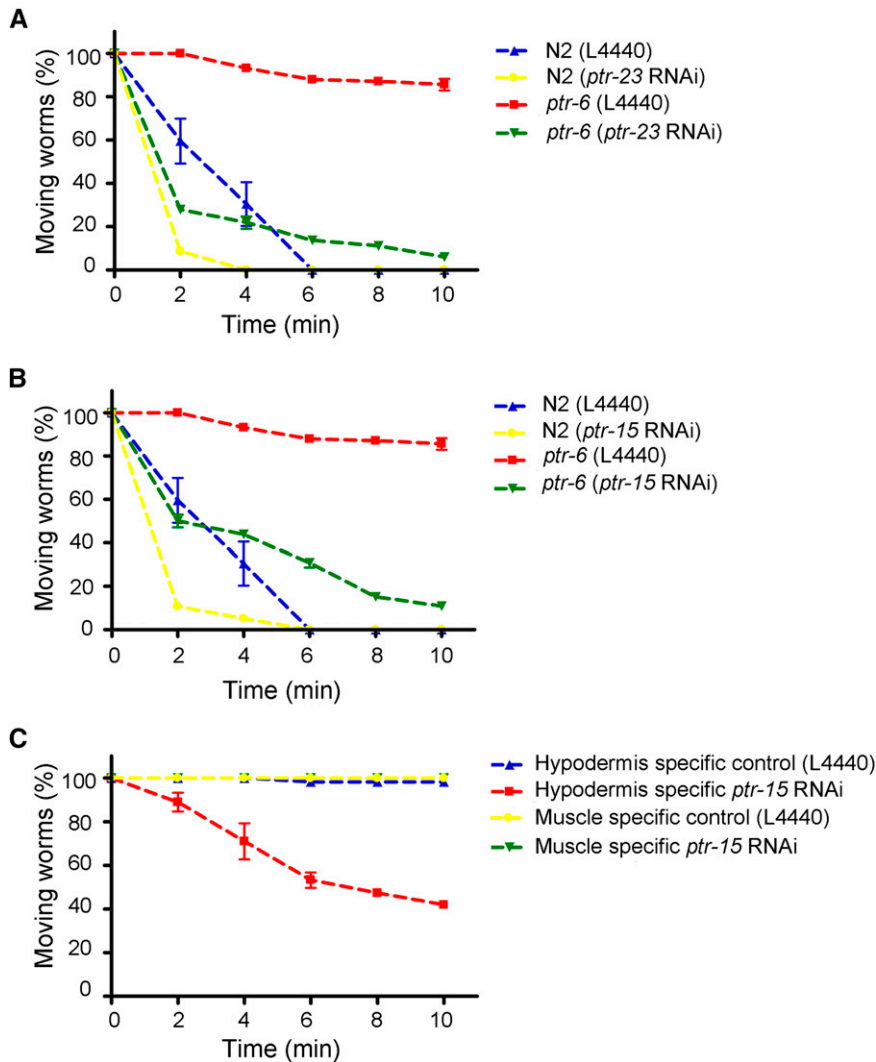


Figure 4 Other *ptr* genes are involved in the regulation of membrane integrity. (A and B) Knock-down of *ptr-15* or *ptr-23* suppressed the ethanol-resistant phenotype of *ptr-6*. (C) Tissue-specific *ptr-15* RNAi experiments using *rde-1* rescue lines show that the site of *ptr-15* action is the hypodermis.

that Patched family protein can regulate trafficking of sterol molecules, which in turn can affect plasma membrane properties.

In this study, we used a high-concentration (7% v/v), ethanol-induced paralysis assay to study membrane integrity. Because 7% (v/v)-induced paralysis is an acute and fast response, the consequence from screening is about ethanol delivery mechanism, not ethanol intoxication. This idea is supported by the facts that the ethanol-resistant mutant in intoxication, *slo-1*, was not isolated by our screening, and that *slo-1(eg142)* null mutants did not show ethanol resistance in our high-concentration assay (data not shown) (Davies *et al.* 2003). The different function of *ptr-6* in the hypodermis and *slo-1* in neurons suggests that the result of drug-resistant mutant screening can generate different targets depending on its concentration and time points. Our previous microarray results based on the same paradigm also support this idea because high concentration of ethanol alters a lot of gene expression, which is involved in lipid metabolism and collagen synthesis (Kwon *et al.* 2004). Based on our results, we

propose the following model for the roles of PTR proteins in membrane integrity: MBOA-1/ACAT synthesizes cholesteryl ester from cholesterol in the hypodermis and PTR-6 acts to regulate the storage of cholesteryl esters in the hypodermis, which leads to changes in membrane permeability. PTR-15 and PTR-23 have roles in regulating membrane permeability in the opposite direction from PTR-6, but it is unclear if they act through the PTR-6-mediated pathway (Figure S6). Among other PTR proteins in *C. elegans*, only PTR-6 has a potential to contain a coiled-coil motif (Thierry-Mieg and Thierry-Mieg 2006). Consistent with our result that PTR-6 is a potential sterol transporter to the cell membrane, there are reports that coiled-coil motifs mediate trafficking in cellular membrane (Knodler *et al.* 2011). Cholesterol and cholesteryl esters are highly associated with membrane thickness (Ma *et al.* 1997) and membrane fluidity (Owen *et al.* 1982). Furthermore, membrane fluidity can be altered by incubating cholesteryl esters with membranes (Kolena and Kasal 1989), supporting our model.

It is also reported that a lipid environment regulates acute functional tolerance (AFT) to ethanol (Bettinger *et al.*

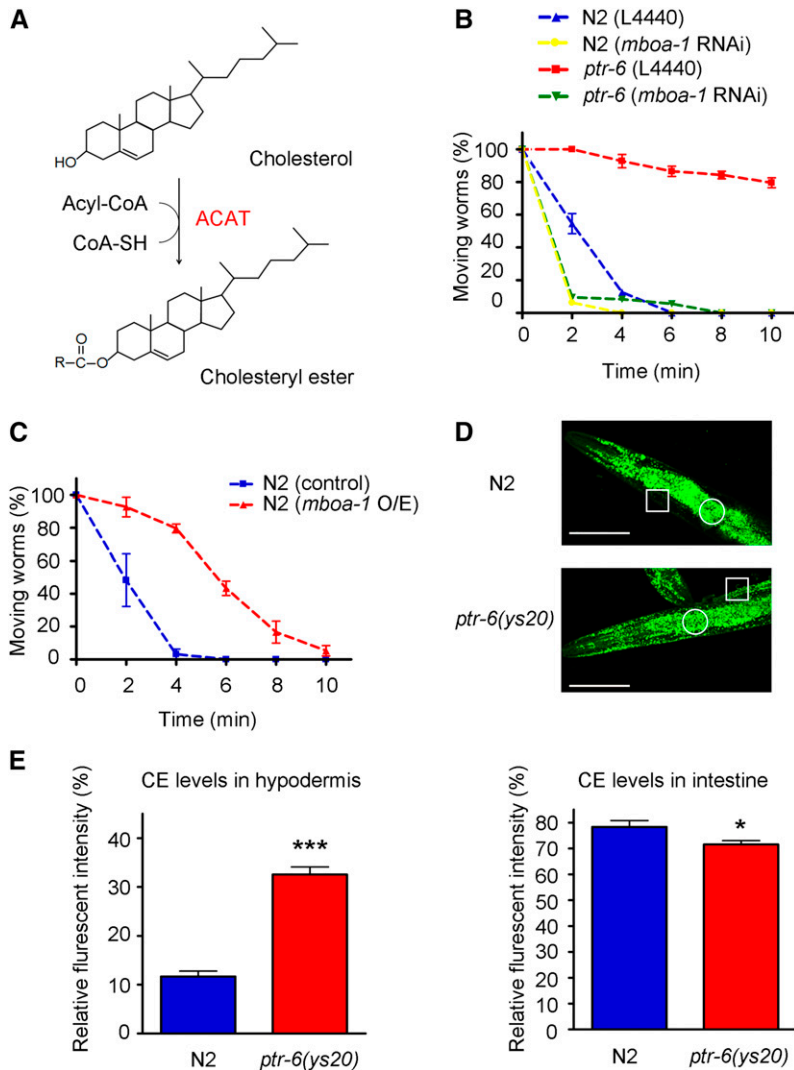


Figure 5 Suppression of *ptr-6* by *mboa-1*, an ACAT homolog. (A) *mboa-1* is a homolog of ACAT, which produces cholesteryl ester using cholesterol and acyl CoA as substrates. (B) *mboa-1* RNAi suppresses the *ptr-6* ethanol-resistant phenotype. (C) *mboa-1* overexpression in the wild-type background causes ethanol resistance. (D) Representative images of cholesteryl ester staining in wild-type and *ptr-6* mutant animals. The intensity of images was measured from the hypodermis or intestine. White rectangles highlight the staining signals from the hypodermis and white circles highlight signals from the intestine. (E) Quantitative analysis of the staining results. *** $P < 0.001$. * $P < 0.05$. Bars, 150 μm .

2012). We think the detailed mechanisms of AFT to 300 mM ethanol and resistance to 1200 mM would be separable because *npr-1*, which is resistant in AFT, is not resistant in our 1200-mM assay. This suggests that the interaction between ethanol and lipid in an organism can be dynamic depending on their concentration and site-of-action.

Cellular membranes are highly vulnerable to many diseases, including metabolic diseases such as atherosclerosis and neurodegenerative diseases. It is notable that the integrity of erythrocytes, which are closely related to membrane integrity, is decreased in atherosclerosis and hyperlipidemia (Stubbs and Smith 1984; Muller *et al.* 1990). Many attempts have been made to attenuate the symptoms of these diseases, and a well-known example is the use of statins (Nicholls *et al.* 2007), which lowers cholesterol levels in the blood, and ACAT inhibitors (Nissen *et al.* 2006b). Symptoms can be also attenuated by lowering dietary cholesterol and inhibiting ACAT (Nissen *et al.* 2006a,b). In this study, we showed that *ptr-6* mutants

show phenotypes that resemble atherosclerosis symptoms, such as rigid membranes. This suggests that *ptr-6* mutant animals can be used as a disease model for atherosclerosis and hyperlipidemia. Interestingly, there was a clinical case where resistance to anesthesia was related to hyperlipidemia in a certain patient (Moore and Smith 2007). According to the report, resistance to anesthesia was secondary to severe hyperlipidemia, which was a side effect of chemotherapy. The *C. elegans ptr-6* mutant may provide a cost- and time-worthy model to screen for new therapeutic treatment of such diseases.

Acknowledgments

Mutant worm strains were kindly provided by the *Caenorhabditis* Genetics Center. The authors thank A. Fire (Stanford University) for vectors. This work was supported by the National Research Foundation of Korea grants funded by the Korean government (no. 2010-0026035 and no. 2013R1A2A03070982).

Literature Cited

- Bettinger, J. C., K. Leung, M. H. Bolling, A. D. Goldsmith, and A. G. Davies, 2012 Lipid environment modulates the development of acute tolerance to ethanol in *Caenorhabditis elegans*. *PLoS One* 7: e35192.
- Bidet, M., O. Joubert, B. Lacombe, M. Ciantar, R. Nehme *et al.*, 2011 The hedgehog receptor patched is involved in cholesterol transport. *PLoS One* 6: e23834.
- Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. *Genetics* 77: 71–94.
- Cases, S., S. Novak, Y. W. Zheng, H. M. Myers, S. R. Lear *et al.*, 1998 ACAT-2, a second mammalian acyl-CoA:cholesterol acyltransferase. Its cloning, expression, and characterization. *J. Biol. Chem.* 273: 26755–26764.
- Chin, J. H., and D. B. Goldstein, 1977 Effects of low concentrations of ethanol on the fluidity of spin-labeled erythrocyte and brain membranes. *Mol. Pharmacol.* 13: 435–441.
- Davies, A. G., J. T. Pierce-Shimomura, H. Kim, M. K. VanHoven, T. R. Thiele *et al.*, 2003 A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell* 115: 655–666.
- Dombek, K. M., and L. O. Ingram, 1984 Effects of ethanol on the *Escherichia coli* plasma membrane. *J. Bacteriol.* 157: 233–239.
- Dupuy, D., Q. R. Li, B. Deplancke, M. Boxem, T. Hao *et al.*, 2004 A first version of the *Caenorhabditis elegans* Promoterome. *Genome Res.* 14: 2169–2175.
- Engelmann, B., S. Streich, U. M. Schonhner, W. O. Richter, and J. Duhm, 1992 Changes of membrane phospholipid composition of human erythrocytes in hyperlipidemias. I. Increased phosphatidylcholine and reduced sphingomyelin in patients with elevated levels of triacylglycerol-rich lipoproteins. *Biochim. Biophys. Acta* 1165: 32–37.
- Gillies, P., and C. Robinson, 1988 Decreased plasma membrane fluidity in the development of atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* 70: 161–164.
- Harrison, R. A., and S. E. Vickers, 1990 Use of fluorescent probes to assess membrane integrity in mammalian spermatozoa. *J. Reprod. Fertil.* 88: 343–352.
- Hausmann, G., C. von Mering, and K. Basler, 2009 The hedgehog signaling pathway: Where did it come from? *PLoS Biol.* 7: e1000146.
- Hobert, O., 2002 PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic *C. elegans*. *Biotechniques* 32: 728–730.
- Hong, M., M. K. Choi, and J. Lee, 2008 The anesthetic action of ethanol analyzed by genetics in *Caenorhabditis elegans*. *Biochem. Biophys. Res. Commun.* 367: 219–225.
- Hunt-Newbury, R., R. Viveiros, R. Johnsen, A. Mah, D. Anastas *et al.*, 2007 High-throughput in vivo analysis of gene expression in *Caenorhabditis elegans*. *PLoS Biol.* 5: e237.
- Ioannou, Y. A., 2001 Multidrug permeases and subcellular cholesterol transport. *Nat. Rev. Mol. Cell Biol.* 2: 657–668.
- Isken, S., and J. A. de Bont, 1998 Bacteria tolerant to organic solvents. *Extremophiles* 2: 229–238.
- Johnson, D. A., N. M. Lee, R. Cooke, and H. H. Loh, 1979 Ethanol-induced fluidization of brain lipid bilayers: required presence of cholesterol in membranes for the expression of tolerance. *Mol. Pharmacol.* 15: 739–746.
- Jung, U. S., and D. E. Levin, 1999 Genome-wide analysis of gene expression regulated by the yeast cell wall integrity signalling pathway. *Mol. Microbiol.* 34: 1049–1057.
- Knodler, L. A., J. A. Ibarra, E. Perez-Rueda, C. K. Yip, and O. Steele-Mortimer, 2011 Coiled-coil domains enhance the membrane association of *Salmonella* type III effectors. *Cell. Microbiol.* 13: 1497–1517.
- Kolena, J., and A. Kasal, 1989 Effects of cholesteryl esters on the accessibility of LH/hCG receptors and membrane lipid fluidity in rat testes. *Biochim. Biophys. Acta* 979: 279–286.
- Kusumi, A., C. Nakada, K. Ritchie, K. Murase, K. Suzuki *et al.*, 2005 Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: high-speed single-molecule tracking of membrane molecules. *Annu. Rev. Biophys. Biomol. Struct.* 34: 351–378.
- Kwon, J. Y., M. Hong, M. S. Choi, S. Kang, K. Duke *et al.*, 2004 Ethanol-response genes and their regulation analyzed by a microarray and comparative genomic approach in the nematode *Caenorhabditis elegans*. *Genomics* 83: 600–614.
- Ma, J., A. R. Folsom, L. Lewis, and J. H. Eckfeldt, 1997 Relation of plasma phospholipid and cholesterol ester fatty acid composition to carotid artery intima-media thickness: the Atherosclerosis Risk in Communities (ARIC) Study. *Am. J. Clin. Nutr.* 65: 551–559.
- Massie, M. R., E. M. Lapoczka, K. D. Boggs, K. E. Stine, and G. E. White, 2003 Exposure to the metabolic inhibitor sodium azide induces stress protein expression and thermotolerance in the nematode *Caenorhabditis elegans*. *Cell Stress Chaperones* 8: 1–7.
- Mello, C. C., J. M. Kramer, D. Stinchcomb, and V. Ambros, 1991 Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* 10: 3959–3970.
- Moore, J., and J. H. Smith, 2007 A case of resistance to anesthesia secondary to severe hyperlipidemia. *Paediatr. Anaesth.* 17: 1223–1225.
- Muller, S., O. Ziegler, M. Donner, P. Drouin, and J. F. Stoltz, 1990 Rheological properties and membrane fluidity of red blood cells and platelets in primary hyperlipoproteinemia. *Atherosclerosis* 83: 231–237.
- Nicholls, S. J., E. M. Tuzcu, I. Sipahi, A. W. Grasso, P. Schoenhagen *et al.*, 2007 Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *JAMA* 297: 499–508.
- Nissen, S. E., S. J. Nicholls, I. Sipahi, P. Libby, J. S. Raichlen *et al.*, 2006a Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. *JAMA* 295: 1556–1565.
- Nissen, S. E., E. M. Tuzcu, H. B. Brewer, I. Sipahi, S. J. Nicholls *et al.*, 2006b Effect of ACAT inhibition on the progression of coronary atherosclerosis. *N. Engl. J. Med.* 354: 1253–1263.
- Owen, J. S., K. R. Bruckdorfer, R. C. Day, and N. McIntyre, 1982 Decreased erythrocyte membrane fluidity and altered lipid composition in human liver disease. *J. Lipid Res.* 23: 124–132.
- Perens, E. A., and S. Shaham, 2005 *C. elegans* daf-6 encodes a patched-related protein required for lumen formation. *Dev. Cell* 8: 893–906.
- Qadota, H., M. Inoue, T. Hikita, M. Koppen, J. D. Hardin *et al.*, 2007 Establishment of a tissue-specific RNAi system in *C. elegans*. *Gene* 400: 166–173.
- Rohlfing, A. K., Y. Miteva, L. Moronetti, L. He, and T. Lamitina, 2011 The *Caenorhabditis elegans* mucin-like protein OSM-8 negatively regulates osmosensitive physiology via the transmembrane protein PTR-23. *PLoS Genet.* 7: e1001267.
- Sikkema, J., J. A. de Bont, and B. Poolman, 1995 Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.* 59: 201–222.
- Solomon, A., S. Bandhakavi, S. Jabbar, R. Shah, G. J. Beitel *et al.*, 2004 *Caenorhabditis elegans* OSR-1 regulates behavioral and physiological responses to hyperosmotic environments. *Genetics* 167: 161–170.
- Stubbs, C. D., and A. D. Smith, 1984 The modification of mammalian membrane polyunsaturated fatty acid composition in

- relation to membrane fluidity and function. *Biochim. Biophys. Acta* 779: 89–137.
- Thierry-Mieg, D., and J. Thierry-Mieg, 2006 AceView: a comprehensive cDNA-supported gene and transcripts annotation. *Genome Biol.* 7(Suppl 1): S12.1–14.
- Wicks, S. R., R. T. Yeh, W. R. Gish, R. H. Waterston, and R. H. Plasterk, 2001 Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. *Nat. Genet.* 28: 160–164.
- Yang, J. S., H. J. Nam, M. Seo, S. K. Han, Y. Choi *et al.*, 2011 OASIS: online application for the survival analysis of lifespan assays performed in aging research. *PLoS One* 6: e23525.
- Zugasti, O., J. Rajan, and P. E. Kuwabara, 2005 The function and expansion of the Patched- and Hedgehog-related homologs in *C. elegans*. *Genome Res.* 15: 1402–1410.

Communicating editor: B. Goldstein

GENETICS

Supporting Information

www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.179705/-/DC1

Maintenance of Membrane Integrity and Permeability Depends on a Patched-Related Protein in *Caenorhabditis elegans*

Myung-Kyu Choi, Sangwon Son, Mingi Hong, Min Sung Choi, Jae Young Kwon,
and Junho Lee

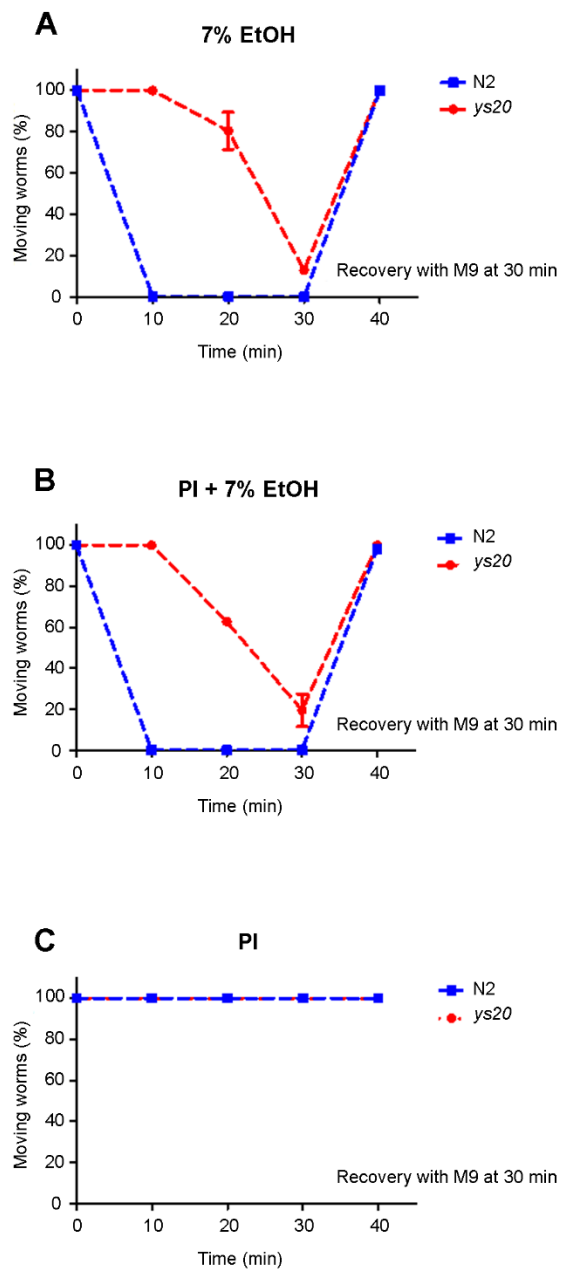


Figure S1 Treatment of 7% ethanol with PI is not a lethal condition

Wild type and *ys20* mutant animals were treated with (A) 7% ethanol, (B) PI with 7% ethanol, and (C) PI for 30 min and moved to M9 buffer for 10 min. All of the animals were recovered.

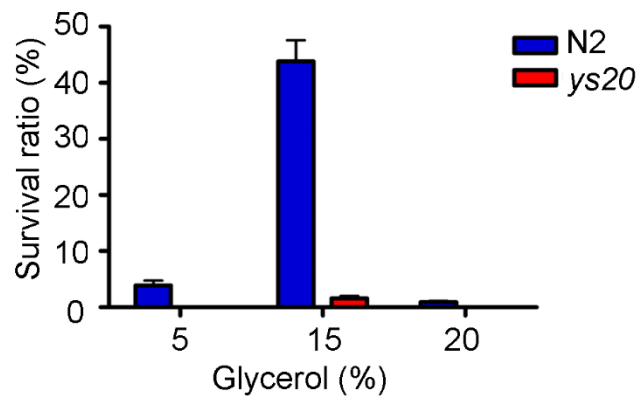


Figure S2 *ys20* animals show low survival rate in freezing solution

Survival ratio of wild type and *ys20* mutant animals after freezing and thawing. The x-axis indicates glycerol concentration in the freezing solution.

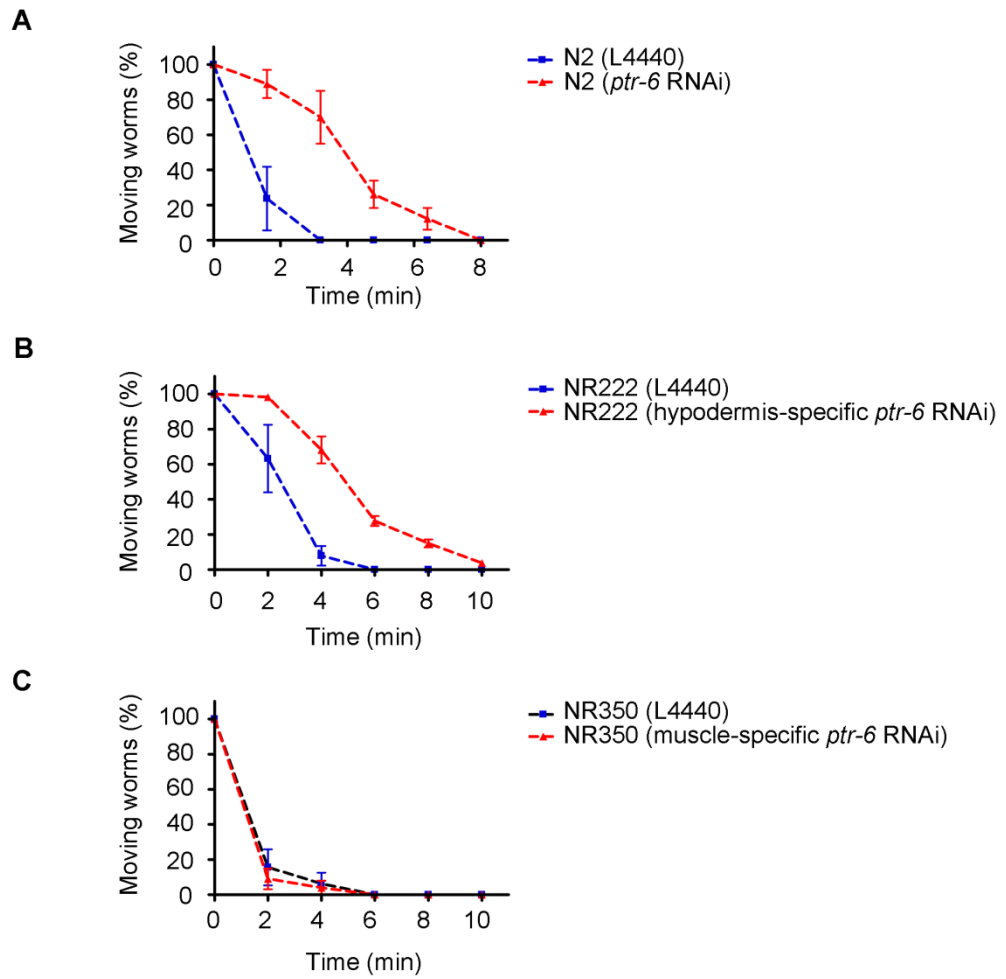


Figure S3 Knockdown of *ptr-6* can induce ethanol resistance phenotype

(A) *ptr-6* RNAi in N2 induces ethanol resistance compared to L4440 control. (B) *ptr-6* is important in the hypodermis for ethanol sensitivity using hypodermis RNAi strains (NR440). (C) Ethanol sensitivity in muscle specific RNAi strain (NR350) of *ptr-6* RNAi experiment was not altered to the L4440 control.

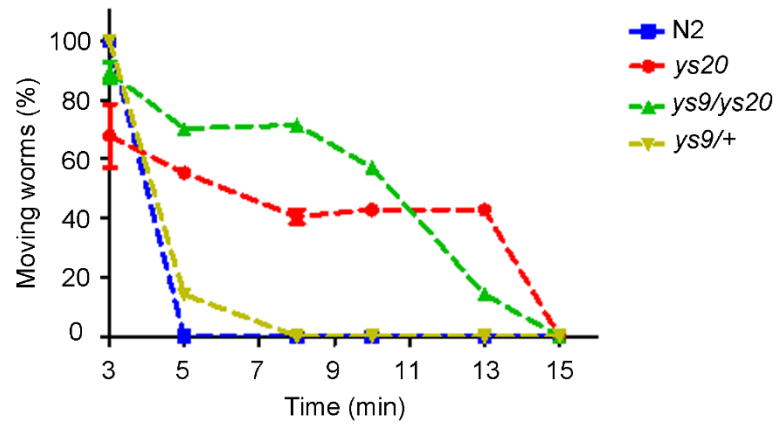


Figure S4 Complementation test between *ys20* and *ys9* mutants

F1 heterozygotes (*ys9/ys20*) were tested in 7% (v/v) ethanol treatments. F1 heterozygote shows similar resistant phenotype with *ys20* mutants.

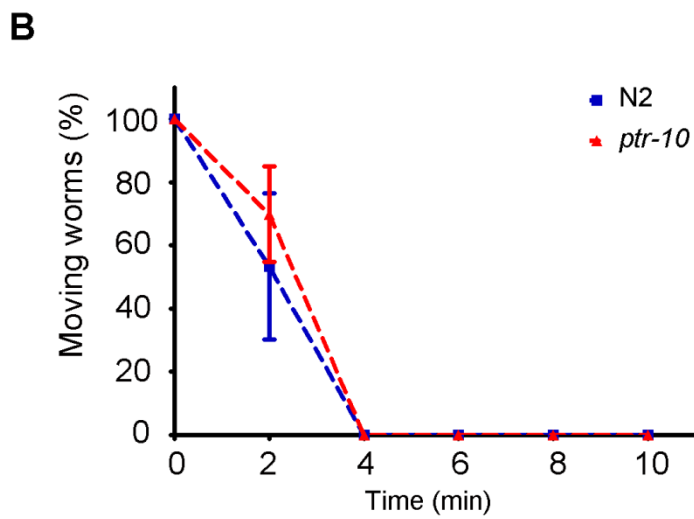
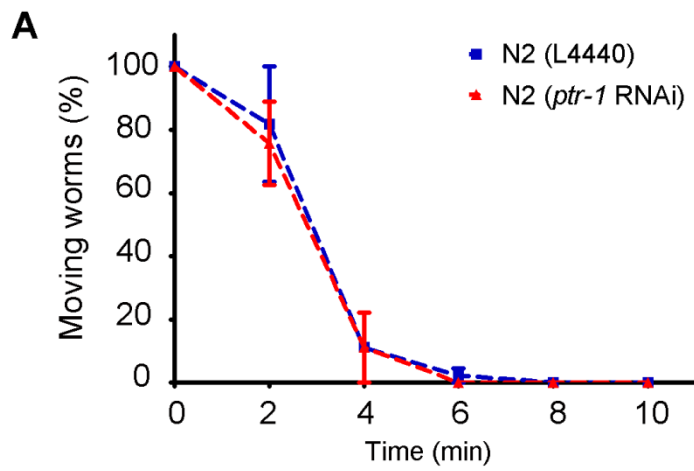


Figure S5 Ethanol sensitivity of *ptr-1* and *ptr-10*

(A) Ethanol sensitivity of *ptr-1* RNAi was similar to that of L4440 control. (B) Ethanol sensitivity of N2 and *ptr-10(ok2106)* was similar.

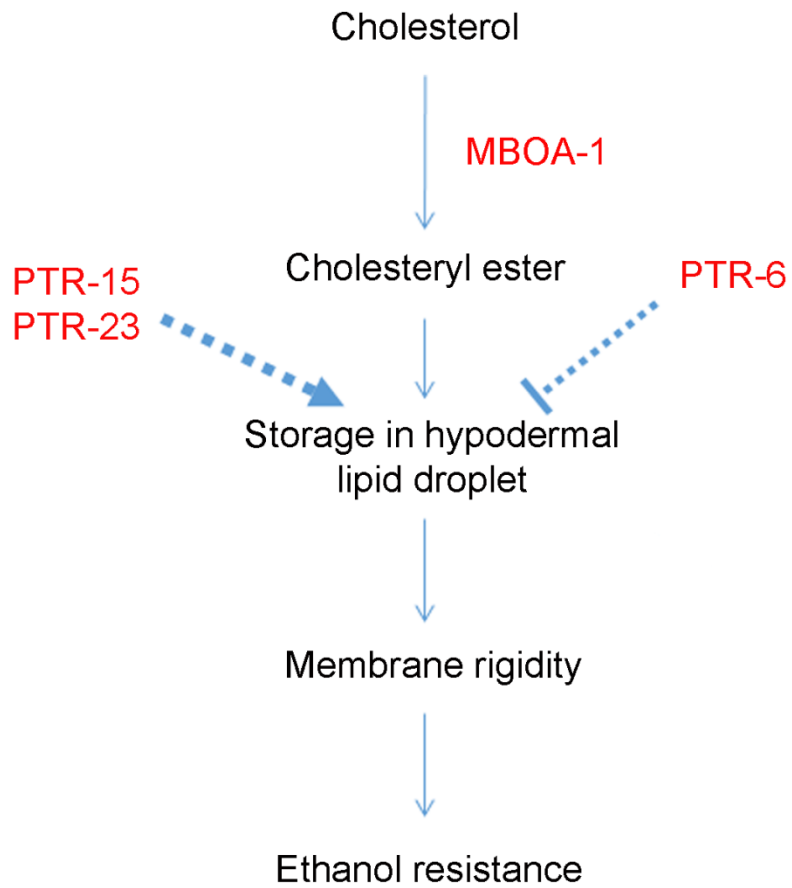


Figure S6 A working model

Regulation of plasma membrane integrity by cholesteryl ester homeostasis and PTR-6. MOBA-1/ACAT synthesizes cholesteryl esters from cholesterol in the hypodermis. PTR-6 may be involved in the maintenance of membrane integrity and fluidity by regulating the trafficking of cholesteryl esters and influencing phospholipid composition.

Table S1 The list of *ptr-6* suppressor RNAi screening experiments

The *ptr-6* suppressor screening is performed to identify all possible genetic interaction of *ptr-6* candidate gene sets were chosen by searching 'cholesterol', 'sterol', or 'steroid' as a keyword in www.wormbase.org and manual inspection.

Sequence name	Gene name	Annotated or predicted function	Ethanol sensitivity
Patched-related family			
F31F6.5	<i>daf-6(ptr-7)</i>	abnormal DAuer Formation;PaTched Related family	Normal
ZK675.1	<i>ptc-1</i>	PaTched family	Normal
F21H12.4	<i>ptc-2</i>	PaTched family	Normal
Y110A2AL.8	<i>ptc-3</i>	PaTched family	Normal
C24B5.3	<i>ptr-1</i>	PaTched Related family	Normal
C32E8.8	<i>ptr-2</i>	PaTched Related family	Normal
C41D7.2	<i>ptr-3</i>	PaTched Related family	Normal
C45B2.7	<i>ptr-4</i>	PaTched Related family	Normal
C53C11.3	<i>ptr-5</i>	PaTched Related family	Normal
C54A12.1	<i>ptr-6</i>	PaTched Related family	Resistant
F44F4.4	<i>ptr-8</i>	PaTched Related family	Normal
F54G8.5	<i>ptr-9</i>	PaTched Related family	Normal
F55F8.1	<i>ptr-10</i>	PaTched Related family	Normal
F56C11.2	<i>ptr-11</i>	PaTched Related family	Normal
K07A3.2	<i>ptr-12</i>	PaTched Related family	Normal

K07C10.1	<i>ptr-13</i>	PaTched Related family	Normal
R09H10.4	<i>ptr-14</i>	PaTched Related family	Normal
T07H8.6	<i>ptr-15</i>	PaTched Related family	Sensitive
T21H3.2	<i>ptr-16</i>	PaTched Related family	Normal
Y18D10A.7	<i>ptr-17</i>	PaTched Related family	Normal
Y38F1A.3	<i>ptr-18</i>	PaTched Related family	Normal
Y39A1B.2	<i>ptr-19</i>	PaTched Related family	Normal
Y53F4B.28	<i>ptr-20</i>	PaTched Related family	Normal
Y65B4BR.3	<i>ptr-21</i>	PaTched Related family	Normal
Y80D3A.7	<i>ptr-22</i>	PaTched Related family	Normal
ZK270.1	<i>ptr-23</i>	PaTched Related family	Sensitive
F46G10.5	<i>ptr-24</i>	PaTched Related family	Normal
Hedgehog-related family			
R08B4.1	<i>grd-1</i>	GRoundHog (hedgehog-like family)	Normal
F46B3.5	<i>grd-2</i>	GRoundHog (hedgehog-like family)	Normal
W05E7.1	<i>grd-3</i>	GRoundHog (hedgehog-like family)	Normal
T01B10.1	<i>grd-4</i>	GRoundHog (hedgehog-like family)	Normal
F41E6.2	<i>grd-5</i>	GRoundHog (hedgehog-like family)	Normal

T18H9.1	<i>grd-6</i>	GRoundHog (hedgehog-like family)	Normal
F46H5.6	<i>grd-7</i>	GRoundHog (hedgehog-like family)	Normal

C37C3.4	<i>grd-8</i>	GRoundHog (hedgehog-like family)	Normal
C04E6.6	<i>grd-9</i>	GRoundHog (hedgehog-like family)	Normal
F09D12.1	<i>grd-10</i>	GRoundHog (hedgehog-like family)	Normal
K02E2.2	<i>grd-11</i>	GRoundHog (hedgehog-like family)	Normal
F02D8.2	<i>grd-12</i>	GRoundHog (hedgehog-like family)	Normal
W06B11.4	<i>hog-1</i>	HOG only (Hedgehog Hog domain alone)	Normal
T05C12.10	<i>qua-1</i>	QUAhog (hedgehog related)	Normal
ZK1290.12	<i>wrt-1</i>	WaRThog (hedgehog-like family)	Normal
F52E4.6	<i>wrt-2</i>	WaRThog (hedgehog-like family)	Normal
F38E11.7	<i>wrt-3</i>	WaRThog (hedgehog-like family)	Normal
ZK678.5	<i>wrt-4</i>	WaRThog (hedgehog-like family)	Normal
W03D2.5	<i>wrt-5</i>	WaRThog (hedgehog-like family)	Normal
ZK377.1	<i>wrt-6</i>	WaRThog (hedgehog-like family)	Normal
ZK1037.10	<i>wrt-7</i>	WaRThog (hedgehog-like family)	Normal
C29F3.2	<i>wrt-8</i>	WaRThog (hedgehog-like family)	Normal
B0344.2	<i>wrt-9</i>	WaRThog (hedgehog-like family)	Normal

ZK1290.8	<i>wrt-10</i>	WaRThog (hedgehog-like family)	Normal
Nuclear hormone receptor family			
F11A1.3	<i>daf-12</i>	abnormal DAuer Formation;a member of the steroid hormone receptor superfamily	Normal
F56E3.4	<i>fax-1</i>	Nuclear Hormone Receptor family	Normal
C48D5.1	<i>nhr-6</i>	Nuclear Hormone Receptor family	Normal
F33D4.1	<i>nhr-8</i>	Nuclear Hormone Receptor family	Normal
C01H6.5	<i>nhr-23</i>	Nuclear Hormone Receptor family	Normal
F11C1.6	<i>nhr-25</i>	Nuclear Hormone Receptor family	Sensitive
Y104H12A.1	<i>nhr-41</i>	Nuclear Hormone Receptor family	Normal
ZK662.3	<i>nhr-48</i>	Nuclear Hormone Receptor family	Normal
K10C3.6	<i>nhr-49</i>	Nuclear Hormone Receptor family	Normal
T09A12.4	<i>nhr-66</i>	Nuclear Hormone Receptor family	Normal
C08F8.8	<i>nhr-67</i>	Nuclear Hormone Receptor family	Normal
W05B5.3	<i>nhr-85</i>	Nuclear Hormone Receptor family	Normal
Y15E3A.1	<i>nhr-91</i>	Nuclear Hormone Receptor family	Normal
T18D3.2	<i>odr-7</i>	Nuclear Hormone Receptor family	Normal
F44A6.2	<i>sex-1</i>	Signal Element on X; nuclear hormone receptor superfamily	Normal
F55D12.4	<i>unc-55</i>	Nuclear Hormone Receptor family	Normal

Cholesteryl ester transferase			
B0395.2	<i>mboa-1</i>	Membrane Bound O-Acyl transferase, MBOAT	Sensitive
H19N07.4	<i>mboa-2</i>	Membrane Bound O-Acyl transferase, MBOAT	Normal
M05B5.4		ortholog of the human gene LECITHIN-CHOLESTEROL ACYLTRANSFERASE	Normal

Lipid binding proteins			
F40F4.3	<i>lbp-1</i>	Lipid Binding Protein	Normal
F40F4.2	<i>lbp-2</i>	Lipid Binding Protein	Normal
F40F4.4	<i>lbp-3</i>	Lipid Binding Protein	Normal
ZK742.5	<i>lbp-4</i>	Lipid Binding Protein	Normal
W02D3.7	<i>lbp-5</i>	Lipid Binding Protein	Normal
W02D3.5	<i>lbp-6</i>	Lipid Binding Protein	Normal
T22G5.2	<i>lbp-7</i>	Lipid Binding Protein	Normal
NPC-1 related proteins			
F02E8.6	<i>ncr-1</i>	NPC1 (human Niemann Pick C disease) Related	Normal
F09G8.4	<i>ncr-2</i>	NPC1 (human Niemann Pick C disease) Related	Normal
Lipoprotein receptors			
F29D11.1	<i>lrp-1</i>	Low-density lipoprotein Receptor related	Normal
T21E3.3	<i>lrp-2</i>	Low-density lipoprotein Receptor related	Normal

Dehydrogenases			
M03A8.1	<i>dhs-28</i>	DeHydrogenases, Short chain	Normal
F56D1.5	<i>dhs-5</i>	DeHydrogenases, Short chain	Normal
C17G10.8	<i>dhs-6</i>	DeHydrogenases, Short chain	Normal
Y6B3B.11	<i>hsd-1</i>	HydroxySteroid Dehydrogenase homolog	Normal

ZC8.1	<i>hsd-2</i>	HydroxySteroid Dehydrogenase homolog	Normal
ZC449.6	<i>hsd-3</i>	HydroxySteroid Dehydrogenase homolog	Normal
C06B3.4	<i>stdh-1</i>	(STeroid DeHydrogenase family	Normal
F11A5.12	<i>stdh-2</i>	(STeroid DeHydrogenase family	Normal
C06B3.5	<i>stdh-3</i>	(STeroid DeHydrogenase family	Normal
F25G6.5	<i>stdh-4</i>	(STeroid DeHydrogenase family	Normal
C56G2.6	<i>let-767</i>	LEThal;steroid dehydrogenase	Normal
Other genes			
C15F1.6	<i>art-1</i>	steroid Alpha ReducTase family	Normal
ZK652.9	<i>coq-5</i>	COenzyme Q (ubiquinone) biosynthesis	Normal
T10B9.10	<i>cyp-13A7</i>	CYtochrome P450 family	Normal
ZK177.5	<i>cyp-44A1</i>	CYtochrome P450 family	Normal
Y57A10C.6	<i>daf-22</i>	abnormal DAuer Formation;ortholog of human sterol carrier protein SCP2	Normal

T13C5.1	<i>daf-9</i>	abnormal DAuer Formation; a cytochrome P450 of the CYP2 subfamily	Normal
B0250.9	<i>dhcr-7</i>	DeHydroCholesterol Reductase	Normal
F55B12.5	<i>nrf-5</i>	Nose Resistant to Fluoxetine; related to mammalian cholesterol-ester-binding proteins	Normal
Y47D3A.17	<i>obr-1</i>	Oxysterol Binding protein (OSBP) Related	Normal
F14H8.1	<i>obr-2</i>	Oxysterol Binding protein (OSBP) Related	Normal

ZK1086.1	<i>obr-3</i>	Oxysterol Binding protein (OSBP) Related	Normal
C32F10.1	<i>obr-4</i>	Oxysterol Binding protein (OSBP) Related	Normal
Y47D3B.7	<i>sbp-1</i>	Sterol regulatory element Binding Protein	Normal
D2013.8	<i>scp-1</i>	SREBP Cleavage activating Protein (SCAP) homolog	Normal
Y44A6D.4	<i>sdf-9</i>	Synthetic Dauer Formation;a protein tyrosine phosphatase	Normal
Y113G7A.11	<i>ssu-1</i>	Suppressor of Stomatin mutant Uncoordination	Normal
Y49E10.11	<i>tat-1</i>	Transbilayer Amphipath Transporters (subfamily IV P- type ATPase)	Normal
H06H21.10	<i>tat-2</i>	Transbilayer Amphipath Transporters (subfamily IV P- type ATPase)	Normal
W09D10.2	<i>tat-3</i>	Transbilayer Amphipath Transporters (subfamily IV P- type ATPase)	Normal
T24H7.5	<i>tat-4</i>	Transbilayer Amphipath Transporters (subfamily IV P- type ATPase)	Normal
K07E3.8	<i>vem-1</i>	VEMA (mammalian ventral midline antigen) related	Normal

C52E4.7		a member of the histidine phosphatase superfamily	Normal
F09C12.8		a member of the histidine phosphatase superfamily	Normal
F53B6.7		a member of the histidine phosphatase superfamily	Normal
F55A11.11		a member of the histidine phosphatase superfamily	Normal
T07F12.1		a member of the histidine phosphatase superfamily	Normal