Clinical Genetics with *C. elegans*

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Clinical case studies are an excellent teaching tool in a genetics course to capture students' attention and introduce them to diseases with a genetic basis. However, students should also learn that the study of genetic diseases in humans has many practical and ethical limitations, necessitating the use of alternative approaches. One historically effective approach has been to study homologous gene function and disease states in model organisms, including the mouse (*Mus musculus*), fruit fly (*Drosophila melanogaster*), and nematode (*Caenorhabditis elegans*), to name a few. This lab is designed to introduce students to these concepts through a three-hour module-based format.

Keywords: case studies, model organisms, *C. elegans*, microscopy, bioinformatics

Introduction

This lab is designed to be accessible to high school students and early undergraduates. There are two stages of preparation for the lab: nematode preparation and day-of preparation. The nematodes must be grown up over the course of several weeks. During this time, slides can be prepared for the lipid accumulation microscopy station. The day-of preparation typically takes one hour, as nematodes must be set up at each of the four microscopy stations. Both stages are described in further detail in the preparation instructions section.

The lab itself is typically presented over three hours, broken down as described in Table 1. For the first hour, we introduce the concept of case studies and then discuss each case study in small groups of students. To wrap up the hour, we review the case studies and segue into model organisms. The second hour focuses solely on the four *C. elegans* microscopy stations, with an opportunity for all participants to observe and interact with mutants defective in locomotion, egg-laying, chemotaxis, and lipid accumulation. At the beginning of the third hour, we take a short break, and then return to discuss the expected results from the four *C. elegans* stations. Finally, we use basic bioinformatics techniques to draw connections between the defective behaviors seen in *C. elegans* and their associated mutated genes, the homologous human genes, and the human diseases caused by mutations in those human genes. Table 2 describes the goals and activities for each module.

Table 1. Timetable for Clinical Genetics with *C. elegans.*

Time	Activity
Hour 1	Introduction (~15 min)
	Clinical Case Studies (~30 min)
	Case Study Review (~10 min)
	Intro to Model Organisms (~5 min)
Hour 2	Worm Doctor, Stations 1-4 (~60 min)
Hour 3	Break (~5 min)
	Review Stations 1-4 (~10 min)
	Bridge with Bioinformatics (~30 min)
	Matching Game (~10 min)
	Lab Wrap-up (~5 min)

Table 2. Module goals, components, and descriptions.

Module	Goals	Stations	Description
Clinical Case Studies	Students will learn to diagnose diseases from clinical case studies.	No stations, work in groups	Identify patient symptoms and test results from each of five case studies in order to diagnose human diseases.
Worm Doctor	Students will use microscopy to observe the four types of *C. elegans* behaviors or phenotypes. Students will attempt to diagnose *C. elegans* "disorders" based on data they collect.	Motor disorders	Observe locomotion in three *C. elegans* strains and illustrate differences.
		Sensing disorders	Observe responses to chemical repellants in two *C. elegans* strains and quantify results.
		Mood disorders	Observe egg-laying behavior in two *C. elegans* strains and quantify results using a line graph.
		Metabolic disorders	Observe lipid accumulation by Oil Red O staining in two *C. elegans* strains and illustrate differences.
Bridge with Bioinformatics	Students will learn to appreciate the utility of model organisms in the study of human diseases.	No stations, work in groups	Use computer databases and programs to identify homologous genes (BLAST) and diseases associated with human genes (OMIM).

1

4

4

Case Study #		
Name	Age	Gender
Clinical symptoms		
Test results		
Diagnosis		
Boston University Clinical Genetics Center (BUCGC) SCI 448, 590 Commonwealth Avenue, Boston, MA 02215. www.bu.edu/lernet/biobugs/		

Part 2: Worm Doctor

Now you will have the chance to run your own tests on your first patients: *C. elegans*! You will rotate through stations 1-4, spending 15 minutes at each one. Follow the instructions and fill in the tables as described.

Station 1: Motor Disorders

Draw what you see under each microscope.

Sample 1	Sample 2	Sample 3

What difference(s) in movement do you notice among the 3 samples?

To practice picking worms, follow these instructions:
1. Make a picking tool by sticking one of your eyebrow hairs to the end of a toothpick using nail polish as the "glue".

2. Practice picking up a worm from the plate labeled "Pick me" and placing it onto the plate labeled "Place me". Try not to dig into the agar when picking and placing.

Station 2: Sensing Disorders
1. Find your specially made osmotic avoidance plates - these plates will have a series of rings drawn on the bottom of them. Label one plate "wildtype" and the other "mutant." Set your timer for 5 minutes.

2. With the help of your lab assistant, pipette a drop of wildtype (WT) worms in the middle of the rings on the "wildtype" plate. Count the number of worms under the microscope.

    Record the WT starting number here: _____

3. Start your timer and watch as the worms move around the plate.

Do the WT worms crawl past the rings on the plates? What does their behavior look like as they approach the rings? Draw pictures or describe below.

4. While your WT worms are crawling, prepare a second osmotic avoidance plate. This time pipette a drop of mutant worms in the middle of the rings. Count the number of worms under the microscope.

    Record the mutant starting number here: _____

5. Start a second timer set for 5 minutes and observe the worms as they move.

        Do you notice any differences between the behavior of the mutant worms and the WT worms when they approach the rings? Draw pictures or describe below.

6. When your timers go off, count the number of worms on each plate that have stayed inside the rings.

    Record the WT number inside the ring here: _____

    Record the mutant number inside the ring here: _____

7. Use the starting total number and the number inside the ring at the end to determine the fraction of worms that avoid the rings:

	WT	Mutant
Number inside ring		
Starting number		
Inside / Starting		

Is there a difference between the fraction of WT and mutant worms that avoid the rings? Which one is better at avoiding?

What do you think is in the rings and why do the worms avoid it? What do you think is wrong with the mutant worms? Talk with your lab assistant about your ideas and find out what was between the rings.

Station 3: Mood Disorders

1. At this station you will find two plates already labeled either "wildtype" or "mutant" that each contain some number of worms on them.

2. Count the number of worms on each plate under the microscope.

   Record the WT number here: _____

   Record the mutant number here: _____

3. After reviewing the sample plate with eggs, count the number of eggs that have been laid on the WT and mutant plates. Record these numbers in the table as count #1 for your round at your station.

4. Wait about five minutes and count the number of eggs a second time. Record these numbers in the table as count #2 for your round at your station.

|	Round 1		Round 2		Round 3		Round 4	
	#1	#2	#1	#2	#1	#2	#1	#2
WT								
Mutant								

Once all groups have completed this station, the numbers from the table will be shared with everyone. Record them in your table above. Use the space below to draw a line graph with "Time" on the x-axis and "Number of Eggs" on the y-axis; this graph could be titled "Number of eggs as a function of time". Be sure to clearly distinguish between your WT line and your Mutant line.

Is there a difference between the number of eggs on the WT and Mutant plates? If so, what could be the reason? Discuss your ideas with your lab assistant and then find out what is wrong with the mutant worms.

Station 4: Metabolic Disorders
    Discuss worm anatomy with your lab assistant. Label the organs in the picture below.
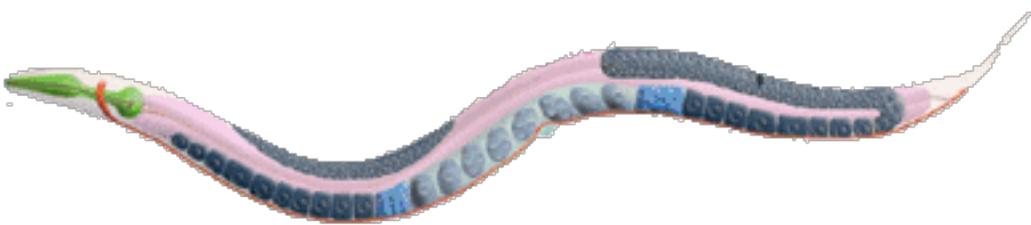
(Reprinted with permission from wormatlas.org)

    There are two slides with worms on them at your station, labeled "WT" and "Mutant". These worms have been stained with a red dye that sticks to lipids, or fats. Using the microscope, look at the worms on the slides.

    What does the fat staining look like in the WT and mutant worms? Draw or describe what you see below.

    Does it look like one set of worms has more staining than the other? Which one? What do you think is the cause of this increase in staining? Discuss your ideas with your lab assistant and find out what is wrong with the mutant worms.

Part 3: Bridge with Bioinformatics

Now you get the chance to see how information stored in databases can help you to draw connections between the mutated genes in worms that cause the "symptoms" you identified, and mutated human genes and their associated diseases.

Work with your lab assistant to go through the following steps for each *C. elegans* gene and fill in the table on the next page.

1. Find Information about the C. elegans Gene and Protein.

Start by heading to the *C. elegans* database called WormBase (http://www.wormbase.org). In the top right corner, there is a search bar where you can search "for a gene." Select one of the genes from the list below and type it into the bar.

A page with information about your gene should appear. On the left hand side of the page, there should be a tool bar where you can select page content. Make sure that "Overview" and "Homology" are selected and then deselect all other content options.

In the Overview section, you can read about what kind of protein your gene encodes and generally how it functions in the worms. **Fill in the table below with a name for the *C. elegans* protein.**

2. Identify the Human Homolog for the C. elegans Protein.

Now scroll down to the Homology section, and look for the table listing "best BLASTP matches." Find the listing in the table for humans (*Homo sapiens*). The "description" column gives you the name of the homologous human protein. **Record the name of the protein in the table below.**

3. Find Disease(S) Associated with the Human Protein.

In a separate browser tab, open the Online Mendelian Inheritance in Man database (http://www.ncbi.nlm.nih.gov/omim). In the search bar, type in the name of the human protein.

Select the first entry in the search results that includes the name of the human protein homolog from Step 2. This will open a page that gives a listing of all known human diseases associated with genetic mutations in your protein of interest. Work with your lab assistant to identify which disease on the list causes symptoms that sound most like one of the clinical case studies from earlier in the day (Part 1). **Write the name of the human disease in the table.**

C. elegans Gene	C. elegans Protein	Human Protein	Possible Disease?
sqt-1			
unc-60			
osm-9			
tph-1			
daf-2			

Match the human diseases discovered in Part 3 to the case studies from Part 1:

Case Study #1 (Joshua) _____

Case Study #2 (Jessica) _____

Case Study #3 (Eddie) _____

Case Study #4 (Eleanor) _____

Case Study #5 (Jayla & Kayla) _____

How many of the patients' diseases were you able to diagnose correctly?

Materials

Computer and projector
Student worksheets (1 per group, 4 groups per session)
Laminated case study cards
Space for 4 groups/stations
(4x) Computers with internet access (1 per group/station)
(7x) Dissecting microscopes, preferably with low wattage/heat bulbs as *C. elegans* are sensitive to high heat over time [(3x) for Station 1: Motor disorders; (2x) for Station 2: Sensing disorders; (2x) for Station 3: Mood disorders]
(2x) Slide microscopes for Station 4
(4x) *C. elegans* life cycle diagram (http://www.wormatlas.org/dauer/introduction/Images/dintrofig1leg.htm) for Station 3
Homemade worm pick materials [1 box of toothpicks, nail polish]
(50x) NGM Agar plates [can obtain from lab-express (http://lab-express.com/plates.htm), Cat No. 5001-60V]
OP50 E. coli
Wildtype (N2) *C. elegans* strain
BE13 [sqt-1(sc1)] *C. elegans* strain
ON19 [unc-60(su158)] *C. elegans* strain
CX10 [osm-9(ky10)] *C. elegans* strain
MT15434 [tph-1(mg280)] *C. elegans* strain
CB1370 [daf-2(e1370)] *C. elegans* strain
[Can obtain OP50 and all *C. elegans* strains from the *Caenorhabditis* Genetics Center (CGC) (https://cgc.umn.edu/)]
(2x) Counters/clickers
10 ml of 4 M Fructose solution
Phosphate buffered saline (PBS)
Microcentrifuge tubes
Disposable plastic transfer pipettes
Glass microscope slides
Glass cover slips
3% Paraformaldehyde in PBS
60% Oil Red O Stain
Microcentrifuge

Notes for the Instructor

This lab was designed in 2016 for the Biology Inquiry and Outreach with Boston University Graduate Students (BIOBUGS) program with a 3-hour timeline in mind. It was delivered as an ABLE 2018 major workshop in a similar format to its original design, with more than half of the focus on the second module, the hands-on experiments with *C. elegans*, and the remaining half of the time dedicated to the first module, the clinical case studies, and the third module, the bioinformatics applications.

Designing the Lab

In order to design this lab, we began by researching phenotypes observed in *C. elegans* that could be easily observed or tested in a classroom setting. We looked for phenotypes affecting mobility, behaviors, and visible growth or development. Some excellent resources for this stage of lab development were WormBook (http://www.wormbook.org/) and WormMethods (http://www.wormbook.org/toc_wormmethods.html). For example, we referenced the chapter on "Obesity and the regulation of fat metabolism" to identify ways to visualize obesity in *C. elegans* (Ashrafi, 2007).

From the resources on *C. elegans* phenoytpes, we were often also able to identify *C. elegans* genes involved in modulating the phenotypes. For example, the Obesity chapter in WormBook describes the gene *daf-2*. In order to learn more about this gene, we searched for *daf-2* in WormBase (https://www.wormbase.org/). In WormBase, we learned from the Overview section that *daf-2* encodes the *C. elegans* insulin receptor, and from the Homology section, we learned that *daf-2* is homologous to the human insulin receptor precursor, INSR.

Finally, we determined whether the human homolog for each *C. elegans* gene was associated with any known diseases or disorders. We searched for the human homolog identified in the WormBase homology table in the Online Mendelian Inheritance in Man (OMIM: https://www.omim.org/) database. For example, we searched for human INSR in OMIM and found that it is associated with several diseases, including Diabetes mellitus.

Hopefully, it has become clear from this description that the lab is actually delivered in a reverse order from how it was originally designed. It is critical to start by identifying genes with known and easily observable phenotypes in *C. elegans* before checking to see if these genes are homologous to disease-causing genes in humans. If one were to start with human diseases and genes, it would likely be much more difficult to (1) find *C. elegans* genes homologous in structure and function, and (2) identify an assay to demonstrate the gene's function that is appropriate for a student laboratory setting. In addition, not all disease-causing mutations in human genes result in visible phenotypes when the same mutations are generated in homologous genes in *C. elegans*. Students may benefit from being reminded during this lab exercise that not all mutations produce visible phenotypes.

One of the unanticipated obstacles of delivering this lab has been maintaining up-to-date information regarding the *C. elegans* genes and proteins. Much of the information was taken from WormBase, which is an actively curated and regularly updated database. When the lab was designed in the Spring of 2016, WormBase was on version WS252, while the site was on version WS264 when the lab was delivered at ABLE 2018, and it is on version

WS267 at the time of submission of these proceedings. With each update, the *C. elegans* community is provided with the most up-to-date information about genes and proteins, which can sometimes mean changes in sequences and homology. These changes resulted in difficulties delivering the lab at ABLE 2018. For example, at the time of designing the lab, the *C. elegans* sqt-1 protein was most closely matched in homology to human Col2A1, a protein associated with Osteoarthritis. As our knowledge of sqt-1 has improved over the years, we have discovered it is better matched with human Col6A5, a protein which is not associated with Osteoarthritis. Thus, this lab must be periodically checked for accurate information and modified when and where needed. Using the description of how the lab was designed, instructors should be able to update the lab with *C. elegans*/human protein pairs that have verifiable phenotypes in worms and have human disease relevance.

Modifying the Lab

There is plenty of built in flexibility in this lab to either expand or consolidate for new applications. The case studies are very flexible – one case study can be assigned per group to decrease time spent on this activity, or each group can be responsible for reviewing all five case studies. Within the worm doctor experiments, there is an opportunity for students to perform the Oil Red O lipid staining themselves instead of having prepared slides to view. This staining protocol adds an additional 30 minutes to the lab length. If this change is made, it is recommended that students rotate through the remaining three worm doctor stations first, and then each of the groups perform the Oil Red O lipid staining concurrently. This option also provides 3-4 sets of Oil Red O stained animals to compare variation between samples. With the bioinformatics module, there is substantial opportunity to add more background information and allow the students more freedom to explore the various databases they are instructed to visit.

The lab is designed to have one or two instructors and four lab assistants. The instructors deliver a presentation designed to introduce students to the core concepts and goals of each module. They are also responsible for keeping track of the timing of the lab. The four lab assistants work directly with the four student groups, guiding the students through each task, asking questions for understanding, and keeping students on track. For the case studies, the lab assistants stay with one group and discuss between 3-5 of the cases, depending on available time. For the worm doctor experiments, each lab assistant is assigned to one of the four stations (motor, sensing, mood, or metabolic). This allows the lab assistant the opportunity to become more familiar with the techniques required for their station and to develop greater background knowledge of their particular worm disorder.

For the bioinformatics applications, the lab assistants can return to their original case study groups to guide the students through the process. The lab assistants are usually graduate students or undergraduates recruited and trained prior to the day of the lab.

Teaching the Lab

The included student worksheet answer key provides some talking points for instructors and volunteers. Additional talking points and information are provided on lab assistant "cheat sheets", which are summarized below.

Module 1: Clinical Case Studies

The goal of this module is to expose students to the life of a clinician. They are provided with a short story about one or two patients and asked to identify the key symptoms and test results. From that information, they are instructed to try to make a diagnosis for their patient(s). There are five case studies and each case has a lab assistant "cheat sheet" that provides additional information about each diagnosis. The lab assistants can use this information to provide more talking points or to gently guide students towards a correct or close diagnosis.

Case Study #1: Nemaline Myopathy

Nemaline myopathy is characterized by muscle weakness, hypotonia, and reduced or absent reflexes. The muscle weakness may also cause difficulty speaking (dysarthria) and swallowing, resulting in feeding difficulties. When discussing this case, we expect that many of the students may not have heard of this disease, so we encourage lab assistants to guide students towards some sort of disease related to motor coordination or the muscles. Perhaps they will mention other similar diseases affecting motor coordination such as Parkinson's, muscular dystrophy, or Hodgkin's disease.

Case Study #2: Depression

The most common symptoms of depression include difficulty concentrating, remembering details, and making decisions; fatigue and decreased energy; insomnia, early-morning wakefulness, or excessive sleeping; irritability, restlessness; loss of interest in activities or hobbies; and overeating or appetite loss. Many students find this case particularly relatable, so lab assistants do not often have difficulty promoting discussion or leading to a correct diagnosis for this case. Students may suggest related disorders affecting mood, including bipolar disorder or seasonal affective disorder.

Case Study #3: Diabetes

There are two types of diabetes, Type I and Type II. Both types of diabetes share numerous characteristics: increased thirst, frequent urination, extreme hunger, irritability and other mood changes, fatigue and weakness,

and blurred vision. Additional symptoms for Type I diabetes are unplanned weight loss; for Type II diabetes they are slow healing wounds and numbness in the extremities. Most students have heard of diabetes, even if they are unaware of exactly how the disease manifests. Given the wide array of symptoms, students may also mention other similar conditions such as Celiac disease, Thyroid disease, or even eating disorders.

Case Study #4: Osteoarthritis

The major symptoms of osteoarthritis are pain and swelling in joints following repetitive use or long periods of inactivity. These symptoms are caused by loss of protective joint cartilage, allowing direct contact between bones. Some students have heard of arthritis, so lab assistants can often guide students towards this diagnosis. Students may also consider other conditions affecting mobility, including rheumatoid arthritis, muscular dystrophy, or fibromyalgia.

Case Study #5: Charcot-Marie-Tooth Disease, Type 2C

The main features of Charcot-Marie-Tooth Disease, Type 2C are leg, arm, and hand weakness and paralysis, vocal chord paresis, diaphragm and intercostal muscle weakness and paralysis. This is a hereditary motor and sensory neuropathy, meaning that there is gradual loss of conduction of signals through axons. Most students will not have heard of CMT Type 2C, so lab assistants are encouraged to guide students towards some sort of sensory disorder, which could include ALS, muscular dystrophy, or myasthenia gravis.

Module 2: Worm Doctor

The goal of this module is to provide students with the opportunity to be "worm doctors": they will perform a number of behavioral and biochemical assays on various *C. elegans* strains to obtain quantitative and qualitative "test results" and identify symptoms. They will use these results to diagnose the worms' diseases. There are four stations in the worm doctor module, investigating motor, sensing, mood, and metabolic disorders. Each station has detailed instructions in the handout for students to follow and a number of questions interspersed that the lab assistants can use to check on student understanding. The lab assistants each receive "cheat sheets" for their specific station, which provide them with tips for the experimental procedures and additional discussion questions.

Station 1: Motor Disorders

For the worm observation portion, microscope #1 will have sqt-1 "roller" worms, #2 will have N2 WT worms, and #3 will have unc-60 "uncoordinated" worms. The students should draw what they see, particularly the differences. They can move the plates around to see different sections of the plate. The lab assistant should help them focus or zoom if they need it.

The lab assistant should help the students find the bunches of unc-60s if the single worms don't look too different from the WT. They can explain to the students that the worms bundle like that because they cannot move far from where they hatch and then they grow up on top of one another.

For the worm picking practice portion, have the students make a worm picker by gluing an eyebrow hair to the end of a toothpick with nail polish. They can try moving WT worms from the "Pick me" plate to the "Place me" plate. They can use the same plates for each round.

Supplementary Questions for Station #1:

Q: Why do you think the worms all move differently?
A: The mutant worms have lost proper muscle function (unc-60, cofilin) or have defects in their connective tissue (sqt-1, collagen II). Both of these mutations cause changes in movement.
Q: Can you think of any human genetic diseases that cause defects in movement?
A: The students might jump to the case studies and the diseases they diagnosed (hopefully osteoarthritis, nemaline myopathy). There are plenty of other diseases with movement defects associated, including Parkinson's, epilepsy, Huntington's, and cerebral palsy (to name a few).

Station 2: Sensing Disorders

Students will find a few worms in the center of a plate that has a ring of a mystery solution (the students are not told initially that the solution is fructose) pipetted onto the surface of the agar. They will monitor the paths of the worms under a dissecting microscope. A majority of the WT worms should avoid the fructose and stay inside the ring. The mutants (osm-9) should not react to the fructose and should crawl across the plate.

Dramatic changes in osmolarity can cause changes in bodily water content in *C. elegans*. High osmotic strength fluids outside of the worm can cause loss of water, making the worms sluggish and decreasing their body mass. The WT worms can sense that a water-soluble repellent with high osmotic strength (fructose) is present and avoid it. The mutant worms have a mutation in a transmembrane receptor that directly recognizes volatile chemicals and odorants, like fructose, so they can't sense the fructose and will not know to avoid it.

Supplementary Questions for Station #2:

Q: Why do think the mutant worms are able to move freely about the plate?
A: See if you can get the students to guess that there is something "smelly" on the plates. Maybe prompt them by asking what makes them avoid going somewhere. Scientific basis described above.

Q: What would happen if we lost our sense of smell?
A: Sometimes it might be a nice thing - can't smell rotten food, body odor, etc. Might also be a sad thing - can't smell freshly baked cookies, flowers, etc. Could also be very dangerous - can't smell smoke from a fire, dangerous chemicals, etc.
Q: Can you think of any human genetic diseases that lead to loss of smell? How about any other senses?
A: Several human diseases have a component of loss of smell (also known as anosmia), including Alzheimer's disease, Parkinson's disease, and multiple sclerosis. For other senses, the students might come back to the case studies and the diseases they diagnosed (hopefully CMT2C, or less specifically the twins and their loss of several different senses).

Station 3: Mood Disorders
There will be three plates: one plate that will be a sample to show what worm eggs look like, one with 5-10 WT worms, one with 5-10 mutant worms (these two plates should have a matched number of worms – the lab assistant should have the students count and record these numbers). After showing the students what eggs look like (this shouldn't take more than a few minutes), have the students count the number of eggs they see on the WT and mutant plate, simultaneously. Have them record their count in the table at the station as count #1 for their round. After a minimum of 5 minutes, have them count again and record under count #2. After 4 rounds, the numbers will be shared and everyone will make a graph of eggs laid over time for both WT and mutants.
The mutant worms should lay fewer eggs than the WT because they have a mutation in tryptophan hydroxylase, an enzyme required to synthesize serotonin. Serotonin is a neurotransmitter that has a role in modulating mood and behaviors. In worms, these behaviors include egg-laying and pharyngeal pumping/eating. Since the mutant worms have less serotonin around, these behaviors are "depressed."

Supplementary Questions for Station #3:
Q: Why do we need to make sure each plate has the same number of worms?
A: This is a variable that can be controlled - if there were more WT worms, then that could be a reason there are more eggs. If there are more mutant worms, then the number of eggs might be the same and the defect would not be recognized.
Q: Why do you think the mutant lays fewer eggs?
A: Talk about circumstances that can cause changes in behavior, both environmental and biological. Focus in on problems with the brain not signaling properly (depression, ADHD, etc.). Scientific basis described above.
Q: Can you think of any human genetic diseases that lead to changes in behavior and mood?

A: The students might jump to the case studies and the diseases they diagnosed (depression). There are plenty of other diseases that cause changes in mood and behavior, including bipolar disorder, anxiety, ADHD, schizophrenia, etc.

Station 4: Metabolic Disorders
C. elegans are very simple invertebrate organisms. Their body plan consists of two tubes (a digestive system and a reproductive system) contained within a third body tube. A majority of worms (>95% naturally occurring) are hermaphrodites, meaning they contain both male and female reproductive organs. Using Figure 1, the lab assistant should help the students label the worm on their worksheet.
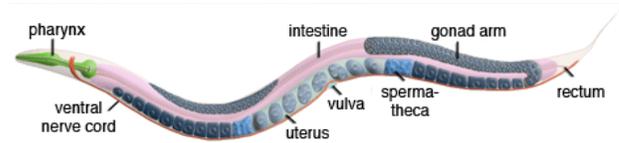

Figure 1. The anatomy of *C. elegans*. Digestive system = pharynx, intestine, rectum; Reproductive system = gonad arm, spermatheca, uterus, vulva; Body tube = musculature, nerves (Reprinted with permission from wormatlas.org)

The lab assistant should help the students look at the stained worms (WT & daf-2 mutants) under the microscope. They should discuss what they see and if they see a difference between the two. Using the diagram, they can identify the part(s) of the worm that seem to have the greatest fat content. The daf-2 mutants should be a much darker/richer red than the WT because they have more fat. These worms accumulate lipids because they have a mutation in their insulin receptor and are essentially insulin resistant. In the worm, insulin resistance promotes lipogenesis.

Supplementary Questions for Station #4:
Q: What part of the worm stores the most fat?
A: The midsection. This is the part of the animal that the intestines run through, where cells should take up glucose from food (or generate lipid stores in a mutant background). Have the students point it out or draw it on their diagram.
Q: Why are the daf-2 mutants more red? (or why aren't they, if not?)
A: They have more fat due to a mutation that doesn't allow cells to respond to insulin properly (scientific basis described above). If the daf-2 mutants do not appear to have more red staining it could be because the staining didn't work right, or the worms were not matched in age, so that the WT worms had been adults for a longer period of time and had accumulated more fat.

Q: Can you think of any human genetic diseases that lead to obesity?
A: The students might jump to the case studies and the diseases they diagnosed (diabetes). There are a number of other diseases that can cause weight gain, including Prader-Willi syndrome, Bardet-Biedl syndrome, Fragile X syndrome, Carpenter syndrome, etc.

Module 3: Bridge with Bioinformatics
The goal of this module is to reinforce for the students the utility of performing scientific experiments using model organisms to learn more about diseases that affect humans. Students have the opportunity to use several computer databases and programs to identify homologous genes (BLAST) and diseases associated with human genes (OMIM). After filling out the chart to identify the human homologs and associated diseases of given *C. elegans* genes, the students are asked to make the connection back to their case studies and give their final diagnoses to their human patients. The lab assistants have the answer keys for this part and are encouraged to guide students towards the preferred disease (some of the human genes are associated with several different diseases). The instructors will wrap up the lab by checking to see how each student group did with their diagnoses and reinforcing the goals of the entire lab.

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Acknowledgments

About the Authors

Melissa LaBonty is an IRACDA Postdoctoral Fellow in the lab of Dr. Bradley Yoder at the University of Alabama at Birmingham. She earned her PhD in Cell, Molecular, and Developmental Biology at Tufts University in 2018. She led the development of this lab for the BIOBUGS (Biology Inquiry and Outreach with Boston University Graduate Students) program at Boston University. Melissa intends to pursue a teaching and research-focused faculty position at a PUI following completion of her postdoctoral fellowship.

Joslyn Mills is a Postdoctoral Research Associate at Brown University in the lab of Dr. Louis Lapierre, where she is studying organellar dynamics associated with aging. She earned her PhD in Cellular and Molecular Physiology from Tufts University in 2018 and worked in collaboration with Boston University to develop her teaching skills and curriculum development.

Angela Seliga has been the Physiology Laboratory Manager at Boston University since 2009, where she divides her time among teaching large laboratory courses in physiology for upper level undergraduate students, training graduate and undergraduate students in pedagogical techniques, and advising students in educational outreach programs.

Renee Johnson is a pharmacy student at the University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences. She completed her BS in Biology and Physics at the University of Colorado Denver in 2014 and her MS in Biomedical Forensic Sciences at Boston University in 2017. While in Boston, she helped design and teach this lab for the BIOBUGS program at Boston University. Renee plans to be a critical care/ER pharmacist upon graduation from pharmacy school in 2021.

Andria Sharma is a first year medical student at the Rutgers New Jersey Medical School. She completed her undergraduate degree in Biology with a specialization in Neurobiology at Boston University in 2016. While in Boston, she aided in the development of this lab for the BIOBUGS program at Boston University.

Feiyuan Yu is currently a PhD student at Boston University, where she helped design this lab. She has been a teaching assistant in a neurophysiological lab since 2016.

Appendix A: Preparation Instructions

Nematode Preparation
~2 months before
1. Obtain worm strains: Wildtype [N2], BE13 [sqt-1(sc1)], ON19 [unc-60(su158)], CX10 [osm-9(ky10)], MT15434 [tph-1(mg280)], QZ91 [daf-2(e1370)]
2. Purchase or make worm plates: Purchase NGM plates from listed supplier or make NGM plates following section 3.2 of the following protocol: http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html#d0e214
Store plates at 4°C.

~1 month before
1. Seed worm plates with *E. coli*: Obtain OP50 *E. coli* from listed supplier. Seed NGM plates with OP50 liquid culture per section 3.3 of the following protocol: http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html#d0e358
2. Once a week, transfer worms from old plates to fresh seeded NGM plates to propagate lines. Follow section 4 of the following protocol: http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html#d0e372

~1-7 days before lab: Oil Red O Staining Protocol
1. Make fresh 60% Oil Red O stain by mixing 3 parts of Oil Red O Stock (5 mg/ml Oil Red O in Isopropanol) with 2 parts of dH2O. Let sit for 10 minutes at room temperature and then filter (note: filters slowly).
2. Obtain plates of WT and daf-2 animals. Wash the worms off each plate into separate microcentrifuge tubes with PBS.
3. Spin the worms down briefly in a centrifuge and then remove the excess liquid. Wash the worms once more with PBS, spin down again, and remove excess liquid.
4. Suspend the worms in 5 drops of 3% paraformaldehyde for 15 minutes. Invert and tap the tube a few times to make sure the worms are immersed in the solution.
5. At the end of 15 minutes, the worms should be settled enough to allow removal of the excess paraformaldehyde. Wash once with PBS, spin down, and remove excess liquid.
6. Suspend the worms in 5 drops of 60% Oil Red O stain and incubate for 60 minutes. Invert and tap the tube a few times to make sure the worms are immersed in the solution.
7. With the worms settled at the bottom of the tube, carefully remove most of the Oil Red O stain. Wash twice with PBS, spinning down and removing excess liquid after each wash.
8. Using a transfer pipet, draw up worms in a small amount of PBS and drop onto a labeled microscope slide and then cover with a coverslip and seal with nail polish to prevent drying.

Day of Lab Preparation Instructions
Part 1: Clinical Case Studies
Distribute three case study cards to each of the four groups, trying to mix them up well enough that each case is covered by at least two groups.

Part 2: Worm Doctor
Station 1: Motor Disorders
1. Prepare healthy worm plates of each genotype (WT, sqt-1, unc-60) by rinsing or picking roughly a dozen worms onto unseeded NGM plates.
2. Set up one plate of each genotype on a dissecting microscope.

Station 2: Sensing Disorders
1. Obtain eight unseeded NGM plates, draw two close rings with a permanent marker on the bottom of the plastic plate, and pipette 100 μl of 4 M Fructose solution between two rings (see pink ring below). Use more Fructose solution if ring does not make full circle.
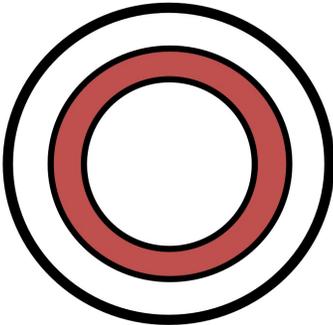
2. Use a small volume (~1 ml) of PBS to wash worms off of plates and into separate microcentrifuge tubes (WT in WT tube, osm-9 in Mutant tube). Worms can hang out in liquid in tube for several hours until used in lab. Just before the students start the station, transfer ~10 WT or osm-9 mutant worms to the center of the plate, within the fructose ring.

Station 3: Mood Disorders
1. Use a small volume (~1 ml) of PBS to wash worms off of plates and into respective microcentrifuge tubes (WT in WT tube, tph-1 in Mutant tube). Worms can hang out in liquid in tube for several hours until used in lab.
2. During case study (Part 1) review, transfer equal numbers of gravid adult WT or mutant worms (aim for 5-10 total) to separate egg laying plates and label accordingly.
3. Make sure to have a plate with eggs on it as a sample so students know what to look for.

Station 4: Metabolic Disorders
Set up prepared Oil Red O stained slides (WT and daf-2) on compound microscopes.

Part 3: Bridge with Bioinformatics
Set up each group with a computer or tablet with internet access.

Appendix B: Evaluations

This lab has been presented once as a part of the BIOBUGS program at Boston University. For the program, classes of up to 24 students from local high schools in the Boston area are invited to attend a lab organized and taught by graduate students. These three-hour labs are offered five times over the span of a week. Clinical Genetics was offered during the fall semester of 2016. Table 3 is a compilation of the students' ratings and comments, as well as comments from the teacher that chaperoned them.

Table 3. Evaluations from BIOBUGS participants.

	Teacher's knowledge of material	Teacher's speaking voice	Teacher's presentation	Lab worksheets	Would they recommend the lab to a friend
Average (Scale 1-5, 5=highest)	4.8	4.5	4.6	4.3	4.4
Student Comments	- "You are wonderful and smart people!" - "I liked being able to use the microscopes on my own" - "I had a great time - "Worms were hard to find" - "I wanted to know more about the worm's habitat" - "Would like information about working in a lab at BU"				
Teacher Comments	- "The amount of support was really impressive" - "Just had an exam on genetics so great extension" - "You were all supportive and encouraging" - "Discussing how a gene sequence leads to gene expression at the beginning would be helpful." - "Need more prompts to participate in the lecture because they do know more but need cues"				

Appendix C: Answer Key

Part 1: Clinical Case Studies

As young doctors, it is important that you record each patient's clinical symptoms and keep track of test results. After you review each case study with your lab assistant, fill in the chart below, making note of the case study number.

Case Study # 1		
Name Joshua	Age 18 months	Gender Male
Clinical symptoms	Unable to walk unassisted, speech impediments, elongated face and high arched palate	
Test results	Weak chest wall muscles with abnormal breathing, rod-like cells in muscle biopsy	
Diagnosis	Nemaline myopathy (students might suggest a motor coordination disorder or disease of the muscles)	

Case Study #2		
Name Jessica	Age 17/18 years	Gender Female
Clinical symptoms	Distracted, unable to concentrate, disorganized, loss of interest in things she used to love, lots of sleep but still fatigued, interrupts/changes topics	
Test results	Decreased brain activity by PET scan Normal ADHD survey scores	
Diagnosis	Depression (students might suggest ADHD, Bipolar, others, hopefully along the lines of a mood disorder)	

Case Study #3		
Name Eddie	Age 16/17 years	Gender Male
Clinical symptoms	Weight loss, insatiable hunger and thirst, fatigue, delayed wound healing	
Test results	Blood: high glucose and low glucose tolerance Urine: glucose present	
Diagnosis	Diabetes (students might suggest Type 1 or Type 2 diabetes - hopefully they agree on some sort of metabolic disorder)	

Case Study #4		
Name Eleanor	Age 40	Gender Female
Clinical symptoms	Joint pain and stiffness, swelling after activities, clicking in her knee, worsening pain over the day	
Test results	Narrowed joint space and bone spur by X-ray, low Vitamin D levels	
Diagnosis	Osteoarthritis (students might suggest broken or fractured bones, some sort of bone disorder)	

Case Study #5		
Name Jayla & Kayla	Age 17 years	Gender Females
Clinical symptoms	J: Can't stand on toes or lift feet at ankles, difficulty breathing after exercise K: Loss of strong voice and high notes, snoring, leg weakness and foot numbness	
Test results	K: Vocal chord paralysis J: Diaphragm paralysis Decrease nerve conduction in limbs, but not in brain	
Diagnosis	Charcot-Marie-Tooth Type 2C (students might not know this name, but hopefully they can guess it is a nerve disorder or a sensing disorder)	

Part 2: Worm Doctor
Station 1: Motor Disorders

  Draw what you see under each microscope.

Sample 1	Sample 2	Sample 3
sqt-1 mutant worms	Wildtype (N2) worms	unc-60 mutant worms
Rollers (rolling in circles)	Normal movement	Uncoordinated, difficulty moving, twitching

What difference(s) in movement do you notice among the 3 samples?

WT worms have smooth movement forward and backwards in a sine/cosine pattern. This movement is what gave the worms the name "elegans," which is Latin for "elegant."

Sqt-1 worms don't move forward very well; they roll around in circles.
(The sqt-1 gene encodes for a cuticle collagen that is important for normal cuticle morphology. The mutant sqt-1 causes left-hand rolling due to defects in the cuticle/outer protective layer of the animal.)

Unc-60 worms hardly move; they twitch, and some worms may be stacked on top of each other because they can't move away from each other.
(The unc-60 gene encodes the actin depolymerizing factor cofilin. Mutant unc-60 causes a lack of actin depolymerization, causing muscles to stiffen and not contract properly and resulting in paralyzed worms.)

Station 2: Sensing Disorders
Do the WT worms crawl past the rings on the plates? What does their behavior look like as they approach the rings? Draw pictures or describe below.

WT worms should stay within the rings.
(The ring contains 4 M fructose, a high osmolarity solution that wildtype animals normally sense as a noxious stimulus and actively avoid.)

Do you notice any differences between the behavior of the mutant worms and the WT worms when they approach the rings? Draw pictures or describe below.

Mutant worms won't stop when they reach the rings
(The osm-9 gene encodes a sensory receptor important for proper response to chemical stimuli, such as 4 M fructose. Osm-9 mutants can't detect the fructose and will move through it unaffected.)

Is there a difference between the fraction of WT and mutant worms that avoid the rings? Which one is better at avoiding?

Hopefully there are more WT worms inside the rings because they can properly detect and avoid the fructose.

What do you think is in the rings and why do the worms avoid it? What do you think is wrong with the mutant worms? Talk with your lab assistant about your ideas and find out what was between the rings.

Mutant worms won't stop when they reach the rings because they have a mutation that doesn't allow them to sense chemicals (in this case, fructose).

Station 3: Mood Disorders

Example Data

|	Round 1		Round 2		Round 3		Round 4	
	#1	#2	#1	#2	#1	#2	#1	#2
WT	1	1	2	3	4	6	7	7
Mutant	0	0	1	1	1	1	2	2
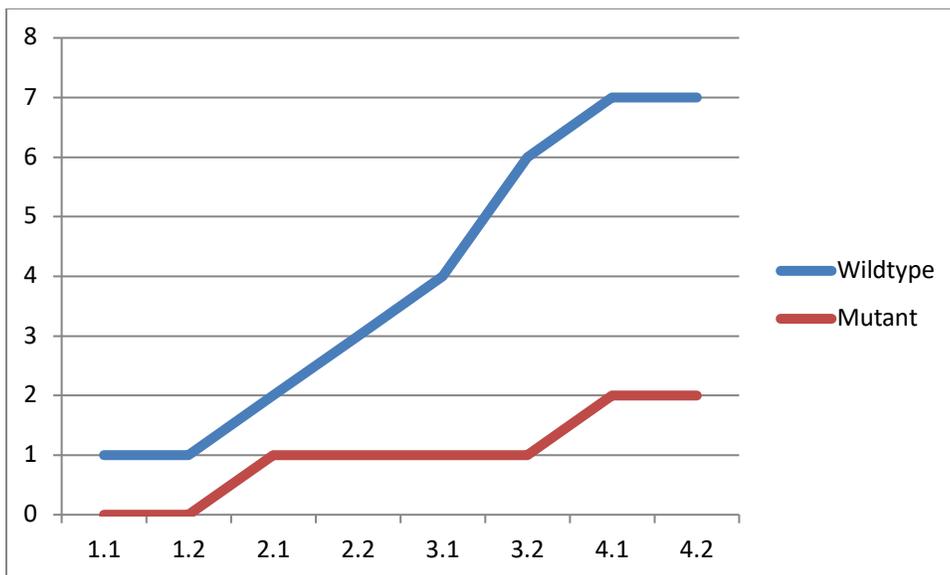
Is there a difference between the number of eggs on the WT and Mutant plates? If so, what could be the reason? Discuss your ideas with your lab assistant and then find out what is wrong with the mutant worms.

WT should have more eggs than the mutant.
(The tph-1 gene encodes a tryptophan hydroxylase indispensable for creating the neurotransmitter serotonin. Mutant tph-1 causes a loss of serotonin and results in "depressed" behaviors, including reduced egg-laying, slowed eating, and increased fat storage.)

Station 4: Metabolic Disorders
Discuss worm anatomy with your lab assistant. Label the organs in the picture below.
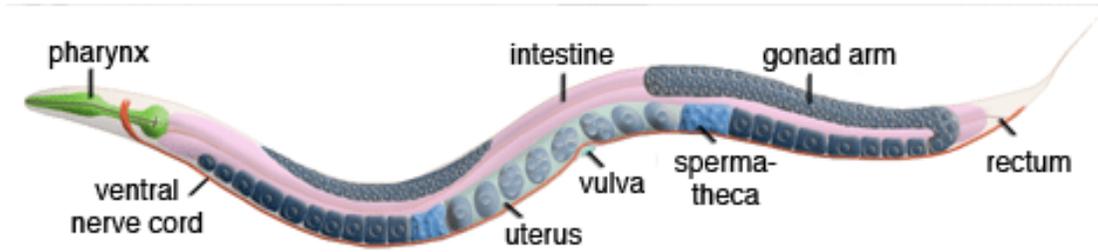
(Reprinted with permission from wormatlas.org)

What does the fat staining look like in the WT and mutant worms? Draw or describe what you see below.

Wildtype   Mutant

Images adapted from Garcia et al., 2015
(Oil Red O stain binds to and marks lipid stores)

Does it look like one set of worms has more staining than the other? Which one? What do you think is the cause of this increase in staining? Discuss with your lab assistant about your ideas and find out what is wrong with the mutant worms.

The mutant worms accumulate lipids and should therefore show much stronger red staining.
(The daf-2 gene encodes an insulin receptor. Mutant daf-2 causes a loss of insulin responsiveness, resulting in lipid accumulation in the worms.)

Part 3: Bridge with Bioinformatics

C. elegans Gene	*C. elegans* Protein	Human Protein	Possible Disease?
sqt-1	Cuticle collagen	Collagen alpha-1(II) / Col2A1	Osteoarthritis
unc-60	Cofilin	Cofilin-2	Nemaline myopathy
osm-9	TRPV channel	TRPV4	Hereditary motor and sensory neuropathy, type IIc / CMT2C
tph-1	Tryptophan hydroxylase	TPH2	Depression
daf-2	Insulin receptor	Insulin receptor / INSR	Diabetes

Match the human diseases discovered in Part 3 to the case studies from Part 1:

Case Study #1 (Joshua) _____**Nemaline Myopathy** _____

Case Study #2 (Jessica) _____ **Depression** _____

Case Study #3 (Eddie) _____ **Diabetes** _____

Case Study #4 (Eleanor) _____**Osteoarthritis** _____

Case Study #5 (Jayla & Kayla) _ **Charcot-Marie-Tooth Neuropathy, Type IIC** _

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