JOURNAL OF EMERGING INVESTIGATORS

Article

Impact of *daf-25* and *daf-11* Mutations on Olfactory Function in *C. elegans*

Jason Gardner, Saman Bhamani, Kendyl Edwards, and Danielle Ereddia
Wheeler High School, Marietta, GA

SUMMARY

A plethora of life-threatening diseases found within humans are thought to result from malformed cilia. The genetic origins of such malformations can be analyzed by testing genes within *C. elegans* that are similar to human cilial genes. In *C. elegans*, chemotaxis and movement toward food are enabled by cilia. Wild-type *C. elegans* were expected to gravitate towards butanone, 2,3-pentanedione, and diacetyl, due to their association with to food, but were expected to move away from 2-nonanone. Chemotaxis assays were used to determine whether mutations in the cilia of two additional strains of nematodes (*daf-11* and *daf-25* mutant *C. elegans*) would alter the chemotaxis indices for these four odorants. Each of the three strains of *C. elegans* were placed on plates with sodium azide and one of the four odorants on either side. Both mutant strains were expected to display difficulty in making an association between the odorants and food due to their malformed cilia; however, we hypothesized that the *daf-25* mutants would be less effective at chemotaxis than the *daf-11* mutants. Our findings partially support this hypothesis. The wild-type *C. elegans* sensed and moved towards the odorants as expected more so than both of the mutant worms, and generally the *daf-11* mutant nematodes appeared to sense and move towards stimulants better than the *daf-25* mutants. There was no statistically significant difference between the senses of the two mutant strains while in the presence of this odorant.

INTRODUCTION

Many human disorders are caused by malformed cilia, such as Bardet-Biedl syndrome, retinopathies, obesity, situs inversus, and polycystic kidney disease (1). The genetic mechanisms behind Bardet-Biedl syndrome have not yet been discovered, but it is classified as a ciliary disorder. In this syndrome, vision loss is very common, along with obesity, delayed development of motor skills and speech, the presence of extra fingers/toes (phalanges), and lower amounts of sex hormones. Kidney abnormalities and loss of smell (anosmia) are also common in Bardet-Biedl syndrome (2). Retinopathy is caused when cilia involved in photoreceptors in the eyes malfunction, which results in vision impairment (3). Situs inversus is a condition where organs are reversed inside the human body. Situs inversus is seen in patients with Kartagener syndrome, which is also known as primary ciliary dyskinesia. Primary ciliary dyskinesia creates abnormal cilia on sperm cells, which can result in situs inversus (4). Cysts in polycystic kidney disease occur when cilia signaling is disrupted due to the malformation of cilia. The abnormal cilia are unable to sense the flow of fluid, which can impact gene expression and cause cysts to form (5).

Two human genes that help facilitate chemosensation have homologs in *C. elegans*. The human gene *NPR2* is homologous to the worm gene *daf-11*. The human gene *ANKMY2* is both homologous and orthologous to the worm gene *daf-25* (6). Mutations in genes that form cilia are responsible for the expression of a plethora of human disease phenotypes, as discussed above (1). These human diseases are greatly impacted by deformed cilia as a result of mutations in the human orthologs of *daf-11* and *daf-25* (2-5). It is unknown what percentage of the diseases mentioned previously are caused by mutations in these genes.

Genetic mutations of the genes in three distinct *C. elegans* neurons lead to the development of abnormal cilia and impaired senses. Similar defects in humans have the potential to cause the aforementioned diseases (1). The three neurons, AWA, AWC, and AWB, are directly related to the nematode's chemosensory abilities. These anterior neurons each correlate with either the repulsion or attraction of *C. elegans* to various odorants that may be present in their environment. AWA and AWC are both necessary for detecting when it is appropriate to move towards a given substance. AWB is necessary for detecting when it is appropriate to move away from a given substance (7). AWC detects the presence of odorants, such as benzaldehyde, butanone, isoamyl alcohol, 2,3-pentanedione, and 2,4,5-trimethylthiazole. AWA detects the presence of diacetyl, pyrazine, and 2,4,5-trimethylthiazole. Both sensory neurons contribute to *C. elegans'* movement towards their respective odorants (7). *C. elegans* are attracted to the aforementioned odorants because the odorants are produced by bacterial metabolism. Even though the worms do not eat the odorants, the smell of the odorants mimics their food because of the way the worms are raised. Therefore, the worms associate the odorants with food and see them as possible nourishment (7). AWB is responsible for their tendency to move away from some odorants, such as 2-nonanone. The reason behind this migration is still unknown (8).

Two genes that are highly relevant to the function of the AWA, AWC, and AWB neurons are *daf-25* and *daf-11*. The mutant strains of the *daf-25* and *daf-11* genes that are being utilized throughout the course of this experiment are *daf-25* (*m362*) and *daf-11* (*m47*). *daf-25* (*m362*) mutants are almost completely ineffective in chemotaxis in response to volatile odorants such as pyrazine, benzaldehyde, isoamyl alcohol, and trimethyl triazole (1). However, *daf-11* (*m47*) mutants are still moderately effective at chemotaxis in response to the volatile odorants listed above (1). Importantly, *daf-11* gene function is dependent on the presence of non-mutated *daf-25* (1).

The *daf-25* and *daf-11* genes result in the attraction or aversion of *C. elegans* towards certain odorants. In this study, we tested the odorants butanone, 2,3-pentanedione, 2-nonanone, and diacetyl. The AWC neuron controls the reaction to the odorants butanone and 2,3-pentanedione, while AWB controls the reaction to the odorant 2-nonanone and AWA controls the reaction to diacetyl. Because of the odorants' affiliation with the three neurons, it is known that the AWC and AWA odorants will be attractants, but that the AWB odorant will be a repellant (7). This new experiment differs from and extends upon a previous study by Jensen and colleagues which included the odorants benzaldehyde, trimethyl triazole, isoamyl alcohol, and pyrazine (1). The first three odorants are detected by the AWC neurons, while the last odorant, pyrazine, is detected by the AWA neuron. The results presented from our experiment have the potential to lend further credibility to the results presented in the previous study by Jensen *et al.*

In the previous study, information was gathered regarding the negative effects that mutant *daf-25* and *daf-11* genes have on the chemosensory capabilities of *C. elegans* while in the presence of pyrazine, benzaldehyde, isoamyl alcohol, and trimethyl triazole (1). This new experiment uses the same mutant genes as the experiment of Jensen *et al.*; however, we tested new odorants to increase the scope of the results. Because the two genes allow for *C. elegans* to have the chemosensory capabilities necessary for everyday life, the mutation of these two genes stifles such capabilities within the worms. Nevertheless, testing the mutant genes using a broader scope of odorants will allow for the hypothesis of this experiment to be tested in greater detail. In addition, further interrogation of the functional role *daf-11* and *daf-25* genes within *C. elegans* will prove helpful to learn more about the diseases and conditions that plague humans. Mutations in genes regulating cilia and their formation are responsible for a plethora of human disease phenotypes, such as the ones mentioned previously.

Our hypothesis was that wild-type worms would be attracted to butanone, 2,3-pentanedione, and diacetyl, resulting in a chemotaxis index close to 1. We also expected that the wild-type worms would be repelled from 2-nonanone, resulting in a chemotaxis index close to negative 1. The mutant worms would also move neither towards nor away from any of the four odorants, resulting in a chemotaxis index close to 0. We expected that the *daf-25* (*m362*) mutation would more greatly inhibit the chemotaxis capabilities of *C. elegans* than the *daf-11* (*m47*) mutation in an extensive number of volatile odorants. As previously stated, mutations of the *daf-25* gene impact the effectiveness and efficiency of both the *daf-25* and *daf-11* genes. Therefore, we expect a mutation in the *daf-25* gene to negatively influence the chemosensory, and therefore chemotaxis, capabilities of *C. elegans* to a greater extent than a mutation in the *daf-11* gene.

RESULTS

We conducted chemotaxis assays to determine the worms' tendencies to move toward or away from a set of given odorants. On petri dishes with Nematode Growth Medium, we pipetted an odorant on one side of the plate, close to the edge. We placed M9 buffer as a control on the opposite side. We drew a line down the middle of the plate, with each sector containing one odorant. We placed a sample of *C. elegans* in the middle of the plate, which represented sample size (Fig. 2). Worms were allowed to migrate to one side for thirty minutes. We counted the worms on each side and calculated a chemotaxis index. We averaged the indices across three trials to generate a final chemotaxis index for each condition (**Figure 1**).

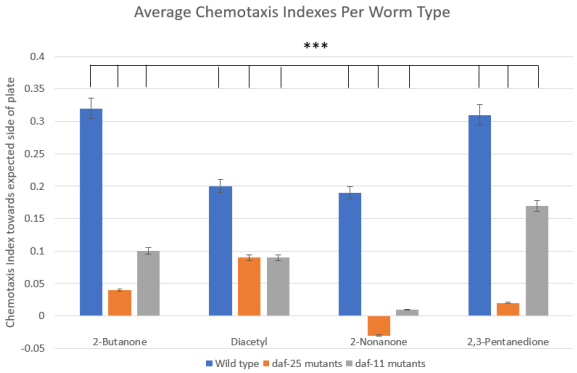

Figure 1. Average chemotaxis indices for each worm type, grouped by odorant. Chemotaxis indices represent how many worms traveled to each side of the plate. An index far from zero indicates a strong preference for that side of the plate, and an index close to zero indicates little preference for either side. n=3 for all tests. *** $p<0.001$ (two-way *ANOVA*).

The average chemotaxis indices are grouped by odorant and display the number of worms that went towards the expected side of the chemotaxis plates. The wild-type indices are higher than those of the *daf-25* and *daf-11* mutants, and the *daf-11* indices are slightly higher than those of the *daf-25* mutants (**Figure 1**). Wild-type worms were attracted to 2-butanone, 2,3-pentanedione, and diacetyl, while mutants were less attracted. Wild-type worms were repelled by 2-nonanone, but the odorant had little impact on the mutant worms. We then performed a two-way *ANOVA* test, a test of variance, on all chemotaxis data. We found that a worm's genotype and the odorant impact results of the chemotaxis assays (p = 0.00096436).

DISCUSSION

The goal of this study was to further the research done by Jensen *et al.* in order to add more significance to their conclusion. As seen in **Figure 1**, the hypothesis was mostly supported. A chemotaxis index close to zero indicates a minimal preference for one side or the other, while an index close to 1 or -1 indicates a strong preference by the group of worms. As hypothesized, the overall results displayed the wild-type nematodes as being significantly more interested than their mutated counterparts in moving towards butanone, 2,3-pentanedione, and diacetyl due to the association they are able to make between the odorants and sustenance. In general, the *daf-25* and *daf-11* mutants yielded indices that were significantly closer to zero than those of the wild-type strain when in the presence of butanone, 2,3-pentanedione, and diacetyl, indicating that they have disrupted cilia function.

Also, for reasons unknown, *C. elegans* are expected to be repelled from the odorant 2-nonanone. This phenomenon

was supported by the wild-type strain data throughout this experiment. The *daf-25* and *daf-11* mutant strains of *C. elegans* also verified claims about the difficulty they had detecting whether it is appropriate to move away from the odorant 2-nonanone, as their indices were -0.03 and 0.01, respectively (**Figure 1**). Our hypothesis, however, was only partially supported. While the differences between wild-type and mutants were as expected, the difference between mutant strains was not anticipated. According to results from previous studies, *C. elegans* that possess a mutated *daf-25* gene are practically ineffective at chemotaxis involving volatile odorants because of the effect a mutation in the *daf-25* gene has on both the *daf-25* and *daf-11* genes within a nematode (1). Results from tests on three out of the four odorants, butanone, 2,3-pentanedione, and 2-nonanone, aligned with previous studies, like those of Jensen *et al.* (1), because of the greater indices the *daf-11* mutants obtained. However, when testing one odorant (diacetyl), the *daf-11* mutant worms did not appear to have better chemotaxis abilities than the *daf-25* mutants, as they were awarded the same positive index: 0.09 (**Figure 1**). This suggests that the chemotaxis abilities of both strains are equal while in the presence of this odorant. It is unknown why this occurred and was most likely a result of a small number of trials. Also shown in **Figure 1**, the *daf-11* mutants obtained a greater index while in the presence of 2-nonanone than the *daf-25* mutants. This suggests that the *daf-11* mutant worms are more adept at identifying when it is appropriate to move away from the 2-nonanone odorant than the *daf-25* mutants. Therefore, the results acquired throughout this experiment partially support the hypothesis that initiated our experiment, as well as the conclusions of various other studies (1).

If this experiment were to be repeated, a few details would be altered. More chemotaxis assays would be completed to increase the statistical power of our results. More trials could increase the validity of the claims we are able to make from our data. Although there are questions of validity concerning the accuracy of manually counting the number of worms on each side of the plate, assuming the counting of worms was accurate, the two-variable *ANOVA* validates our data and deems it statistically significant. However, additional trials could verify our hypothesis further and confirm our results. Another element that could be improved in later experiments would be the aseptic technique throughout this experiment, such as sterilizing workspaces more often and with greater care. Taking greater precaution when performing this experiment could result in more accurate data. The last improvement for future experiments would be to utilize kimwipes to wick away excess M9 buffer. Some worms were rendered immobile due to excess M9 buffer, which could have altered their chemotactic ability and therefore the data.

This experiment's data informs many future studies. Existing protocols may be expanded upon in the future by testing the result of new volatile odorants, such as benzaldehyde, isoamyl alcohol, and 2,4,5-trimethylthiazole. One could also conduct this experiment with a dual mutant strain of *daf-25* and *daf-11*, as this may have an impact on a strain's response to different odorants. Lastly, testing a *daf-19* strain would also be helpful, as this strain regulates many cilia-related genes and is critical in the development of sensory cilia (1). Discovering more about the role mutations play in the chemosensory abilities of *C. elegans* will allow humans to gain a greater understanding of how mutations of chemosensory-regulating genes may impact their lives.

METHODS

Nematode Growth Media Plate Preparation

A 1-liter Erlenmeyer flask was used to mix the solution for the NGM agar, 3 g NaCl, 17 g agar, 2.5 g peptone, and 975 mL H_2O. It was then autoclaved for 50 minutes and cooled in a 55°C water bath for 15 minutes. After it was cooled, 1 mL 1M $CaCl_2$, 1 mL 5 mg/mL cholesterol, 1 mL 1 M $MgSO_4$, and 25 mL 1 M KPO_4 buffer were added. It was then mixed by swirling and subsequently poured into 6-cm petri dishes until they were ⅔ full (9).

Bacterial Preparation

A single colony of *E. coli* OP50 was selected from a plate and grown in LB overnight at 37°C with agitation. An L-rod was then used to transfer OP50 to the center of 15 NGM plates. They were incubated at room temperature for 48 hours, and then stored upside down at 4°C or were used immediately (10).

Worm Growth

A worm sample was added to a pre-prepared NGM plate containing *E. coli*. This plate was then left alone for 4–7 days. M9 Buffer was used to transfer worms to a new plate that already contained *E. coli*. The plate was incubated at room temperature for two days. This was done to give the worms a constant food source since their given mutations prevent dauer formation.

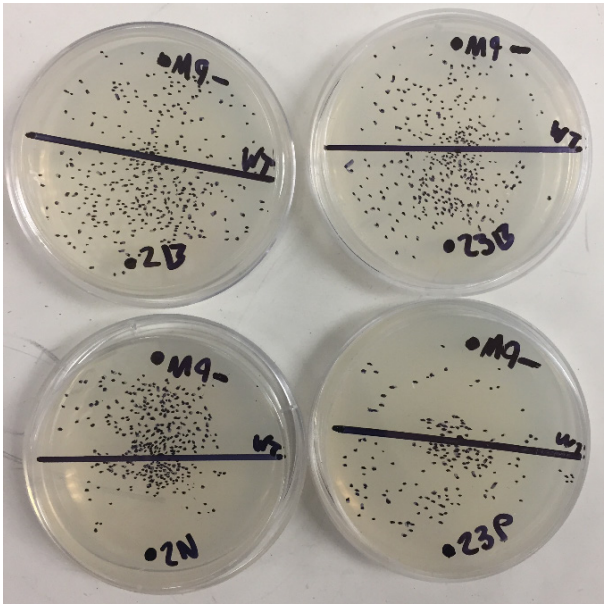

Figure 2. Chemotaxis plates for trial 1 of wild-type worms. An image of the four chemotaxis plates from trial 1 of the wild-type chemotaxis assays. It shows that the worms were attracted to butanone, 2,3-pentanedione, and diacetyl, but repelled by 2-nonanone.

Washing Worm Samples

A pipette was used to transfer 1 mL M9 buffer on the plate containing the subculture of *C. elegans*. The plate was tilted

until the whole surface was covered and was left to sit for 30 seconds. Afterwards, the plate was tilted at a 45° angle to allow *C. elegans* to pool at the edge of the plate. A pipette was then used to transfer this liquid into a microcentrifuge tube. After the *C. elegans* settled at the bottom, the supernatant was removed from the top, and 1 ml M9 buffer was added back into the microcentrifuge tube. This wash step was repeated two more times, but on the last time, approximately 200 µL M9 buffer was left in the tube above the *C. elegans* (11).

Chemotaxis Assay

First, a pre-made NGM plate was labeled and divided into a control side and a chemical side. Then, 2 µL 0.5 M sodium azide was added to the outermost spots on opposite sides of the plate. This immobilized the worms once they reached the far sides of the plate. About six hours later, 2 µL of the designated odorant was added to the test side of the plate, and 10 µL of M9 buffer was added to the control side. A micropipette was used to transfer 4 µL of the *C. elegans* sample from the microcentrifuge tube with the same label. The excess M9 buffer was absorbed using a tissue so as to not hinder the worms' movement. After 10 minutes passed and it was ensured that the *C. elegans* were traveling across the agar, they were left at room temperature for 30 minutes face up. The plates were held against a bright light and worms were counted on their respective sides, and a chemotaxis index was calculated (**Figure 2**). Each chemotaxis index was calculated by subtracting the number of *C. elegans* on the control side from the number of *C. elegans* on the odorant side. This number was then divided by the total number of worms on that plate. A chemotaxis index was calculated for every plate that contained one of the three strains of *C. elegans*, as well as one of the four odorants used during this experiment. "Worm Growth," "Washing Worm Samples," and "Chemotaxis Assay" were repeated for each odorant and *C. elegans* strain (11).

Data Analysis

A two-variable *ANOVA* was utilized to determine the significance of the results acquired throughout the experiment. A two-variable *ANOVA* recognizes the influence two independent variables may have on the dependent variable of an experiment. For this particular experiment, the independent variables are the three strains of worms (wild-type *C. elegans*, *daf-11* mutant *C. elegans*, and *daf-25* mutant *C. elegans*) and the four odorants (butanone, 2,3-pentanedione, diacetyl, and 2-nonanone), while the dependent variable is the chemotaxis index. The indices are based on the chemotaxis abilities each strain of *C. elegans* has in the presence of four odorants.

Received: January 04, 2018
Accepted: November 17, 2018
Published: February 2 , 2019

REFERENCES

1. Jensen, Victor L., *et al.* "Localization of a Guanylyl Cyclase to Chemosensory Cilia Requires the Novel Ciliary MYND Domain Protein DAF-25." *PLoS Genetics*, vol. 6, no. 11, 2010, doi:10.1371/journal.pgen.1001199.
2. "Bardet-Biedl Syndrome - Genetics Home Reference - NIH." U.S. National Library of Medicine, *National Institutes of Health*, Accessed: 15 May 2018, ghr.nlm.nih.gov/condition/bardet-biedl-syndrome#definition.
3. Ghosh, Amiya K., *et al.* "Human Retinopathy-Associated Ciliary Protein Retinitis Pigmentosa GTPase Regulator Mediates Cilia-Dependent Vertebrate Development." *Human Molecular Genetics*, vol. 19, no. 1, 2009, pp. 90–98., doi:10.1093/hmg/ddp469.
4. Guichard, Cécile, *et al.* "Axonemal Dynein Intermediate-Chain Gene (DNAI1) Mutations Result in Situs Inversus and Primary Ciliary Dyskinesia (Kartagener Syndrome)." *The American Journal of Human Genetics*, vol. 68, no. 4, 23 Feb. 2001, pp. 1030–1035., doi:10.1086/319511.
5. Yoder, B. K. "Role of Primary Cilia in the Pathogenesis of Polycystic Kidney Disease." *Journal of the American Society of Nephrology*, vol. 18, no. 5, 2007, pp. 1381–1388., doi:10.1681/asn.2006111215.
6. Zerbino, Daniel R, *et al.* "Ensembl 2018." *Nucleic Acids Research*, vol. 46, no. D1, 16 Nov. 2017, doi:10.1093/nar/gkx1098.
7. Bargmann, Cornelia. "Chemosensation in C. Elegans." *WormBook*, 25 Oct. 2006, doi:10.1895/wormbook.1.123.1.
8. Troemel, Emily R, *et al.* "Reprogramming Chemotaxis Responses: Sensory Neurons Define Olfactory Preferences in C. Elegans." *Cell*, vol. 91, no. 2, 17 Oct. 1997, pp. 161–169., doi:10.1016/s0092-8674(00)80399-2.
9. Stiernagle, Theresa. "Maintenance of C. Elegans." *WormBook*, 11 Feb. 2006, doi:10.1895/wormbook.1.101.1.
10. Porta-De-La-Riva, Montserrat, *et al.* "Basic Caenorhabditis Elegans Methods: Synchronization and Observation." *Journal of Visualized Experiments*, no. 64, 10 June 2012, doi:10.3791/4019.
11. Hart, Anne C. "Behavior." *WormBook*, 3 July 2006, doi:10.1895/wormbook.1.87.1.