8 Genetic Testing Unit Assessment: *ALAD* and *SOD1*

Introduction

As an assessment of the unit, students revisit some of the bioinformatics tools they have used in prior lessons to locate a mutation in a protein associated with a harmful genetic condition. Students also evaluate current genetic tests for the condition using the criteria of clinical validity and treatment options. Two conditions and their tests are presented: porphyria and amyotrophic lateral sclerosis (ALS).

Learning Objectives

At the end of this lesson, students will know that:

- Genetic tests vary in terms of utility.
- Similar bioinformatics and bioethical tools can be used when assessing the utility of different genetic tests.

At the end of this lesson, students will be able to:

- Apply bioinformatics tools for comparing and visualizing information to a new genetic testing example.
- Evaluate a genetic test in terms of its clinical validity, available treatment options, and any related ethical considerations.

Key Concepts

- Bioinformatics tools can be used to compare sequences and identify differences.
- Visualization software can help researchers understand how genetic changes impact protein structure.
- Mutations can impact the ability of a protein to fulfill its function, which can in turn result in disease.
- Genetic tests vary in terms of clinical validity and available treatment options.

Class Time

One class period (50 minutes).

Prior Knowledge Needed

- Completion of *Lessons One* through Six.

Common Misconceptions

- All genetic tests are similar in their utility.
- All genetic disorders are similar in their modes of inheritance.

Materials

Materials	Quantity
Class set of Student Handout—*Porphyria*	1 per student for half of class (class set)
Class set of Student Handout—*Amyotrophic Lateral Sclerosis*	1 per student for half of class (class set)
"Amino Acid Abbreviations and Chemistry Resources" (Optional for Challenge Questions, found in the *Appendix*)	1 per student
Teacher Answer Key—*Porphyria*	1
Teacher Answer Key—*Amyotrophic Lateral Sclerosis*	1

Computer Equipment, Files, Software, and Media
Assessment protein sequences can be downloaded from http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing. Provide local copies for students, or ask them to download the sequences directly.
Lesson Eight PowerPoint® Slides—Bioinformatics and Genetic Testing. Available for download at: http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing.
A student version of lesson materials (minus teacher answer keys) is available from NWABR's Student Resource Center at: http://www.nwabr.org/students/student-resource-center/instructional-materials/introductory-bioinformatics-genetic-testing.
Computer lab with internet access for students, the program Cn3D, and a word processing program such as Microsoft Word® or Google Docs for answering the assessment questions.

Teacher Preparation

- Make copies of the Student Handouts, one per student, with half of the class working on porphyria, and half of the class working on ALS. Alternatively, the entire class could work on the same condition, or pairs of students can be assigned to work on different conditions together. These handouts are designed to be reused as a class set.
- Teachers will need to provide the protein sequences for ALS and porphyria, both the reference sequence and the patient sequence for each disease. These sequences should be in an electronic format in a central location where students will have access to them during class, or they can be accessed directly from the NWABR website. These sequences can be found at: http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing.
- A PowerPoint® presentation for *Lesson Eight*, which includes the answer key for both activities, is posted on the curriculum website. This can be used to review students' answers.

Procedure

1. Explain to students the ***aim of this lesson***. Some teachers may find it useful to write the aim on the board.

   Lesson Aim:

   - Apply what you have learned about genetic testing to a new genetic disorder.

   Teachers may also wish to discuss the Learning Objectives of the lesson, which are listed at the beginning of this lesson plan.

2. Remind students that they have learned about many aspects of genetic testing, including: 1) comparing patient DNA sequences to reference sequences to identify mutations; 2) viewing the three-dimensional structure of a protein to determine where a mutation is located and what effect a particular mutation may have on the function of the protein; and 3) considering the ethical issues involved in genetic testing.

3. Explain to students that in today's lesson, they will be applying each of these skills to the study of a different genetic disease: either porphyria or Amyotrophic Lateral Sclerosis (ALS).

4. Either assign students to one of the two diseases (porphyria or ALS), allow students to pick one of the diseases to study, or assign pairs of students to work on different conditions together.

5. Pass out Student Handout—*Porphyria* to the porphyria group(s) and Student Handout—*Amyotrophic Lateral Sclerosis* to the ALS group(s). Students will need to use computers with internet access.

6. Tell students to read the background information about their assigned genetic condition before beginning the activities in *Parts I*, *II* and *III*.

7. Tell students where to find the electronic versions of the patient and reference protein sequences for both genetic disorders, as well as the protein structure files.

8. Students will perform a BLAST alignment first using the protein sequences (*Part I*), then examine the protein structure and identify where the mutation is located (*Part II*), and last, discuss the ethical considerations of genetic testing for their assigned condition (*Part III*). For *Parts I* and *II*, students will document their work with screen capture images that they will save in a word processing document, along with their answers to the questions from Student Handout—*Porphyria* or Student Handout—*Amyotrophic Lateral Sclerosis*.

9. Students should turn in the word processing document with their completed answers, via email or print. The assignment could also be finished as homework.

[**Note:** Scientists use the one letter amino acid abbreviations for protein sequences. (See the *Appendix*, "Amino Acid Abbreviations and Chemistry Resources," for a list of the one letter amino acid codes and chemistries, if needed).]

Closure

10. Tell students that in today's lesson, they applied what they had learned in earlier lessons to a genetic condition that was new to them. All genetic testing involves comparing DNA or protein sequences obtained from patients to reference sequences, as students did with BLAST. If the structure of the protein is known, as in the case of porphyria and ALS, scientists and doctors can locate the mutation on the protein structure to try to determine how that mutation might affect the function of the non-mutated protein. It is important to realize that not all genetic tests are equally useful, and not all genetic disorders are inherited in the same way. For example, most cases of ALS are not hereditary, and there is no effective treatment. ALS is usually caused by a sporadic mutation, so genetic testing and family pedigrees are not likely to be useful. In contrast, porphyria is hereditary and there are effective treatments available, so genetic testing would be useful.

11. Students have seen throughout these lessons that many different people, representing many different types of careers, make genetic testing possible. These include:
    - The ***bioengineers*** who design the machines like the DNA sequencer.
    - The ***genetic counselors*** and ***veterinarians*** who work directly with human and animal patients.
    - The ***laboratory technicians*** who collect and sequence patient samples.
    - The ***3D animators*** who help us represent and understand massive amounts of biological data.
    - The ***bioethicists*** who help us understand the ethical implications of genetic testing, for ourselves, our families, and our communities.

    Direct-to-consumer genetic testing offers individuals greater access to their genetic information, but it is not without its challenges and risks. As science and technology continue to advance, today's high school students face a future full of increasing amounts of genetic information. What they choose to do with it will be up to them.

Extension

- Challenge students to use their understanding of amino acid chemistry and the program Cn3D to develop a hypothesis about how their particular mutation might impact protein binding or some other function.
- Challenge students to research nucleotide and protein sequences for a disease or disorder of their choosing, and try to find an associated protein structure. In some cases, the protein structure may have been solved for only part of the protein. As students saw with the BRCT domain of BRCA1, while only part of the BRCA1 protein structure is known, there is still much to learn about how mutations can impact the protein's function.
- Facilitate a discussion about the pros and cons of direct-to-consumer genetic testing. Ask students to reflect upon whether their views about direct-to-consumer genetic testing have changed since they were first exposed to the topic in *Lesson One*.

Glossary

Amyotrophic Lateral Sclerosis (ALS): A progressive, fatal neurodegenerative disease that results in the degradation of motor neurons.

Free radicals: Highly reactive molecules capable of causing tissue damage and enhancing the effects of aging.

Heme: An iron-containing molecule that forms the non-protein portion of hemoglobin and is essential for the transport of oxygen by hemoglobin.

Hemoglobin: A protein responsible for transporting oxygen in the blood of vertebrates.

Heterogen: Any molecule that is not part of a DNA, RNA, or protein chain, such as a metal ion.

Motor neurons: Nerve cells that form the pathways for signals that pass along the brain and spinal column.

Porphyria: A rare hereditary disease in which the blood pigment hemoglobin is abnormally metabolized. Porphyrins are excreted in the urine, which becomes dark. Other symptoms include mental disturbances and extreme sensitivity of the skin to light.

Credit

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BBC News, King George: Mad or Misunderstood? Accessed 1/29/10 from: http://news.bbc.co.uk/2/hi/3889903.stm.

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History of Porphyria. Accessed 1/29/10 from: http://www.porphyriafoundation.com/about-porphyria/history-of-porphyria/.

King George image from Wikimedia Commons. http://en.wikipedia.org/wiki/File:George_III_in_Coronation_Robes.jpg.

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Stephen Hawking. Accessed 10/4/10 from: http://en.wikipedia.org/wiki/Stephen_Hawking.

Stephen Hawking image from Wikimedia Commons. Accessed 10/4/10 from: http://en.wikipedia.org/wiki/File:Stephen_Hawking.StarChild.jpg.

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8 Porphyia (Poor-fear-E-ah)

(From *porphyrus*, meaning purple, a reference to the darkly colored urine of patients with porphyria)

Background

Photo Credit: Wikimedia Commons. http://en.wikipedia.org/wiki/File:George_III_in_Coronation_Robes.jpg.

King George III, who ruled England at the time of the American Revolution, was termed "mad" because of his bizarre and unusual behavior. His symptoms (severe pain in his abdomen, delirium, confusion, dark red urine) were not understood at the time. Some historians believe that his medical condition – which was not diagnosed until the 1970s as porphyria – may have influenced his ability to rule the colonies. If so, porphyria may be one reason that the United States became a separate country!

Porphyria is related to a defect in the manufacture of **heme**, an important part of **hemoglobin** (the oxygen carrier in red blood cells). Each molecule of the protein hemoglobin contains four heme groups, which are the site of oxygen binding.

There are at least eight types of porphyria, and they are very different from each other. However, in all porphyrias molecules that are precursors to heme build up in the body. There are eight steps in making heme from the amino acid glycine, and each step requires a different enzyme. If there is a mutation in one of these enzymes, too many precursors could be made, and not enough heme. This is the case when there are mutations in the gene *ALAD*, the second step in the path to making heme.

Most of the symptoms of porphyria impact the nervous system or skin, and are not very specific (such as blistering or burning of areas of skin exposed to the sun). There is no cure for porphyria, but each type of the disease has treatments available (such as the heme therapy "Panhematin"). Mild attacks are treated with intravenous glucose, and a high carbohydrate diet is recommended for patients.

Heme: An iron-containing molecule that forms the non-protein portion of hemoglobin and is essential for the transport of oxygen by hemoglobin.

Hemoglobin: A protein responsible for transporting oxygen in the blood of vertebrates.

DNA testing for porphyria will detect most of the known disease-causing mutations. If mutations are detected, it can provide relief to families to know that the symptoms are related to porphyria rather than a mental illness.

Although labor-intensive and expensive, DNA testing is very reliable. However, many different genes could cause porphyria, so often multiple genes need to be tested. There are no common mutations so each possible gene must be completely sequenced in each new family.

PART I: BLAST Protein Alignment

ALAD is a gene that encodes one enzyme in the pathway to making heme. Take the reference ALAD protein sequence and use BLAST to align the amino acid sequences of the two proteins to determine where the mutation is (similar to Student Handout—*Aligning Sequences with BLAST Instructions* from *Lesson Four*).

1. Go to the NCBI BLAST page: http://blast.ncbi.nlm.nih.gov/Blast.cgi.
2. Select "**protein blast**."
3. Check the box "**Align two or more sequences**." A second text box will appear.
4. Copy the ALAD Reference sequence into the first box and the patient ALAD sequence into the second box. Be sure to include the "**>**" symbol.
5. Click "**BLAST**."
6. Once your search is complete, click the "**Formatting options**" link, and use the drop-down menu under the "**Alignment View**" to select "**Query-anchored with dots for identities**." Click the "**Reformat**" button in the upper right corner.
7. Open your word processing software and create a new document. You will use this document for recording your answers and saving screen images while you work. Label the document your LASTNAME_ALAD_NCBI. Type your name, class period, and date at the top of the blank page. Save the document once you have pasted in your alignment.
8. Capture an image of the aligned sequences from the BLAST results page and paste it in your document. Add a descriptive title to describe the image and save your document.
9. Answer the following questions under your screen shot in your document:

   Are there any differences between the reference (query) sequence and the patient sequence? If so, answer the following:

   a. Specify where the change is by describing its location by number within the protein (for example, at position 181, etc.).
   b. Use the one-letter amino acid abbreviations to describe which amino acid has changed, and what the new amino acid is. (For example, M has changed into R).
   c. Describe whether the change is a substitution (one amino acid exchanged for another), an insertion (an amino acid where there was none before), a deletion, or some other kind of change.
10. Answer the following question in several complete sentences: How can tools such as BLAST help scientists study and treat genetic diseases?

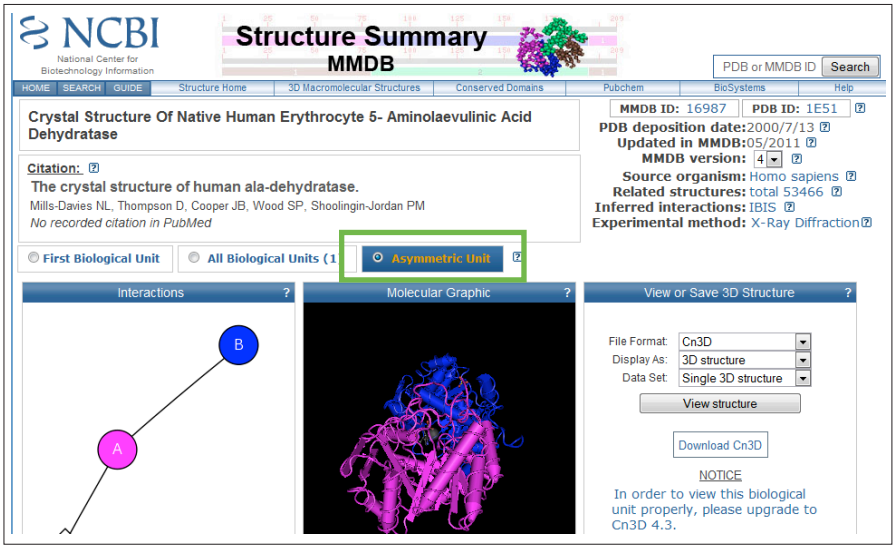

Figure 1: Select the "Asymmetric Unit." Credit: NCBI Structure, Molecular Modeling Database (MMDB).

PART II: Protein Visualization Using Cn3D

In *Part II*, you will visualize both the reference protein and the location of the mutation in a mutant protein using the Cn3D program. First, we're going to investigate the structure of a functional protein (1E51). Then, we will look at a structure from a protein with the same mutation we identified in *Part I* (1PV8).

11. Go to the NCBI structure page: http://www.ncbi.nlm.nih.gov/Structure/index.shtml.
12. In the search box, type the structure accession number "**1E51**" and click "**Go**."
13. Click on the picture of the structure or the structure title to go to the Structure Summary.
14. Select the option "**Asymmetric Unit**" in the middle of the screen (see ***Figure 1***).
15. Click "**View structure**" and open the file in Cn3D.

    [**Note:** depending on your browser, you may have to download the file and then open it in Cn3D.] Notice that there are two identical chains of amino acids that interact with each other (1E51_A and 1E51_B), one of which is colored pink and the other blue.
16. In the Sequence Viewer window, move your cursor until you come to the position of the mutation identified in *Part I: BLAST Protein Alignment*. (For example, if you found in *Part I* that the mutation was at position 8, you would move your cursor over the amino acids until you saw that you were over amino acid 8 in the bottom left window of the Sequence Viewer.) Be sure to confirm that you are looking at the correct amino acid by double-checking the sequence of the amino acids immediately before and after the mutation. There is often more than one of each type of amino acid in a protein sequence.
17. Hold down the "**CONTROL**" key ("**Command**" on Mac) and click to select the amino acid in that position in both chains in the Sequence Viewer. The individual amino acids that you just selected should be highlighted yellow.
18. Turn the structure around to see where the mutation is located relative to the two protein chains.
19. In your word processing document, in one to two sentences, describe where the mutation is located in the protein structure.
20. Experiment with different "**Style -> Rendering Shortcuts**" and zoom in and out until you find an image that you think shows the mutation location well relative to the overall shape of the protein (such as ball and stick or spacefill).
21. Capture the image of this protein structure with the location of the mutation highlighted, insert it into your word processing document, and type a descriptive title for your image.
22. Return to the NCBI structure page and find and open the structure "**1PV8**," making sure to select the asymmetric unit before opening the structure, as you did with the previous protein. 1PV8 is the structure of the mutant protein.
23. Use the same "**Rendering Shortcuts**" as you did with the reference protein structure.
24. Highlight the mutation location as you did with the reference protein structure.
25. Rotate the protein so that it is oriented in approximately the same way as the reference protein structure image, to see how the mutation affects the shape of the protein.
26. Capture the image of the mutated protein structure, insert it into your document, and type a descriptive title for your screen image.

27. Answer the following questions under your screen images of the reference and mutated protein structures in your document:
    a. In general, how can mutations impact the function of a protein?
    b. How might the specific mutation that you looked at impact the protein?
    c. Answer the following in a few complete sentences: How can tools such as Cn3D help scientists study and treat genetic diseases?

Challenge: Look up the full names of the amino acids involved in the mutation using the "Amino Acid Abbreviations and Chemistry Resources" showing one-letter abbreviations. How are they chemically different? How might this difference impact the protein's function?

Optional: Use your drawing tools in Word® to use an ***arrow*** to point out the location of the mutation in the sequence alignment in the BLAST sequence window and in the Cn3D view. ("**Insert Shapes -> Arrow -> Color**"). If you prefer, you can draw ***red circles*** instead. ("**Insert Shapes -> Oval -> Shape Fill: no fill, Shape outline: red line**"). If you want to crop the image, you can use the ***Crop*** feature in Word®.

PART III: Genetic Testing

Use the information in the background section, as well as the knowledge you gained from the genetic testing lessons in this unit, to ***answer the following questions in your word processing document:***

28. How clinically valid is the genetic test? (Low, Medium, or High) Explain why you chose that level.
29. Is there an effective treatment for this condition, whether medical or behavioral? Explain your answer.
30. If you were a genetic counselor, and this condition was found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?
31. If you were a genetic counselor, and this condition was not found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?
32. What are the characteristics of a good genetic test, in your opinion? To what extent do direct-to-consumer genetic tests that consumers can purchase on their own meet your criteria?

Turn in your Microsoft Word® document in the manner specified by your teacher.

8 Amyotrophic Lateral Sclerosis (ALS)

(**ah**-my-uh-tro-fik **lah**-tuh-rul skluh-**ro**-sis)

Background

Stephen Hawking, a highly regarded theoretical astrophysicist, has a condition called Amyotrophic Lateral Sclerosis (ALS). ALS causes progressive loss of neuromuscular control. Despite his physical challenges, Hawking served as Professor of Mathematics at the University of Cambridge for thirty years. He is best known for his influential theories on black holes and quantum gravity and his popular book, *A Brief History of Time*. Hawking was one of the youngest people elected to the British Royal Society, and won the 2009 US Presidential Medal of Freedom. Hawking uses a voice synthesizer that converts his typed messages into speech.

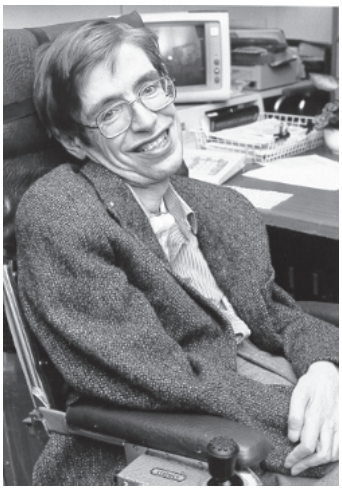

Photo Credit: Wikimedia Commons. http://en.wikipedia.org/wiki/File:Stephen_Hawking.StarChild.jpg.

Amyotrophic means that the muscles have lost their nourishment (from Greek, "a" = without, "myo" = muscle, "trophic" = nourishment). ***Lateral*** refers to the sides of the spinal cord, where the nerves that interact with muscles are found ("lateral" = side). ***Sclerosis*** means the diseased portion of the spinal cord becomes hardened or scarred ("sclerosis" = hardening or scarring).

ALS affects nerve cells in the brain and the spinal cord. The **motor neurons** that reach from the brain to the spinal cord and from the spinal cord to the muscles in the body degenerate and eventually die. As a result, brain signals can no longer start and control muscles. In later stages of the disease, patients may become paralyzed.

ALS is hereditary in only a small percentage of families; 90% of individuals with adult-onset ALS do not have a family history of the disease. Such individuals have "sporadic" ALS. There is a genetic component to this type of ALS but it is not inherited; it arises during an individual's development. Because most individuals do not have hereditary ALS, ALS is usually diagnosed by a doctor who reviews a person's symptoms and medical tests. When ALS is inherited (in 10% of cases), it is usually inherited in an autosomal dominant manner. Changes in a gene on chromosome 21, superoxide dismutase (*SOD1*), have been found in about 20% of families with inherited ALS. The SOD1 protein normally detoxifies **free radicals**, molecules that are harmful to cells. Somehow, changes in SOD1 result in harm to motor neurons. Both prenatal and pre-symptomatic tests for *SOD1* changes related to ALS exist. However, most inherited ALS (80%) is not due to changes in *SOD1*, and currently there are no other genetic tests to offer. Inherited (or "familial") ALS is usually diagnosed based on family history. The two types of ALS do not differ in terms of symptoms. A positive genetic test does not change the way that ALS is treated, but it may allow families to plan for the future more effectively. However, a positive genetic test does not indicate precisely when someone with ALS will begin to show symptoms. Other genes in addition to *SOD1* are involved in ALS, but tests have not been developed for these genes. So an individual who has a family history of ALS but tests negative for mutations in *SOD1* may still develop ALS.

Motor neurons: Nerve cells that form the pathways for signals that pass along the brain and spinal column.

Free radicals: Highly reactive molecules capable of causing tissue damage and enhancing the effects of aging.

Scientists have developed a way to study ALS using mice, based on changes in the *SOD1* gene. The mouse model helps them understand how mutations in *SOD1* can lead to the symptoms of the disease, and allows them to look for new treatments. Currently, there are no preventative treatments for ALS, only life-prolonging ones.

[**Note:** Amyotrophic Lateral Sclerosis is sometimes called "Lou Gehrig's Disease," although some evidence suggests that Lou Gehrig may have had a different condition.]

PART I: BLAST Protein Alignment

SOD1 is a gene that encodes the protein superoxide dismutase. Take the reference SOD1 protein sequence and use BLAST to align the amino acid sequences of the two proteins to determine where the mutation is (similar to Student Handout—*Aligning Sequences with BLAST Instructions* from *Lesson Four*).

1. Go to the NCBI BLAST page: http://blast.ncbi.nlm.nih.gov/Blast.cgi.
2. Select "**protein blast**."
3. Check the box "**Align two or more sequences**." A second text box will appear.
4. Copy the SOD1 Reference sequence into the first box and the patient SOD1 sequence into the second box. Be sure to include the "**>**" symbol.
5. Click "**BLAST**."
6. Once your search is complete, click the "**Formatting options**" link, and use the drop-down menu under the "**Alignment View**" to select "**Query-anchored with dots for identities**." Click the "**Reformat**" button in the upper right corner.
7. Open your word processing software and create a new document. You will use this document for recording your answers and saving screen images while you work. Label the document your LASTNAME_SOD1_NCBI. Type your name, class period, and date at the top of the blank page. Save the document once you have pasted in your alignment.
8. Capture an image of the aligned sequences from the BLAST results page and paste it in your document. Add a descriptive title to describe the image and save your document.
9. Answer the following questions under your screen shot in your document:

   Are there any differences between the reference (query) sequence and the patient sequence? If so, answer the following:

   a. Specify where the change is by describing its location by number within the protein (for example, at position 181, etc.).
   b. Use the one-letter amino acid abbreviations to describe which amino acid has changed, and what the new amino acid is. (For example, M has changed into R).
   c. Describe whether the change is a substitution (one amino acid exchanged for another), an insertion (an amino acid where there was none before), a deletion, or some other kind of change.
10. Answer the following question in several complete sentences: How can tools such as BLAST help scientists study and treat genetic diseases?

PART II: Protein Visualization Using Cn3D

Visualize both the reference protein and the location of the mutation in a mutant protein using the Cn3D program. First, we're going to investigate the structure of a functional protein (1HL5). Then we will look at a structure from a protein with the same mutation we identified in *Part I* (1OEZ).

11. Go to the NCBI structure page: http://www.ncbi.nlm.nih.gov/Structure/index.shtml.

12. In the search box, type the structure accession number "**1HL5**" and click "**Go**."

13. Click on the picture of the structure or the structure title to go to the Structure Summary.

14. Click "**View structure**" and open the file in Cn3D.

    [**Note:** depending on your browser, you may have to download the file and then open it in Cn3D.]

    Notice that there are two chains of amino acids that interact with each other. ***Also note the many metal ions that are important for SOD1 activity (Cu, copper and Zn, zinc).***

15. In the Sequence Viewer window, move your cursor along 1HL5_A until you come to the position of the mutation identified in *Part I: BLAST Protein Alignment*. (For example, if you found in *Part I* that the mutation was at position 8; you would move your cursor over the amino acids until you saw that you were over amino acid 8 in the bottom left window of the Sequence Viewer.) Be sure to confirm that you are looking at the correct amino acid by double-checking the sequence of the amino acids immediately before and after the mutation. There is often more than one of each type of amino acid in a protein sequence.

16. Hold down the "**CONTROL**" key ("**Command**" on Mac) and click to select the amino acid in that position in both chains in the Sequence Viewer. The individual amino acids that you just selected should be highlighted yellow.

17. In your word processing document, in one to two sentences, describe where the mutation is located in the protein structure.

18. Open "**Style -> Edit Global Style**" and click the "**Labels**" tab. Check the box beside "**Metal ion labels**," if it is not checked already, then click "**Apply**" and "**Done**."

19. Open "**Select -> Select by Distance**."

> **Heterogen:** Any molecule that is not part of a DNA, RNA, or protein chain, such as a metal ion.

20. A window will appear with the default distance. Click the box near "**Select heterogens**" and click "**OK**."

21. Experiment with different "**Style -> Rendering Shortcuts**" and zoom in and out until you find an image that you think shows the mutation location well relative to the overall shape of the protein (such as ball and stick or spacefill).

22. Zoom in closer to the protein structure to look at the mutation site and the nearby metal ions, rotating the protein to see how a mutation at that site might impact the interaction of the protein with a metal ion. [**Hint:** Which metal ion is colored yellow?]

23. Capture the image of this protein structure with the location of the mutation highlighted, insert it into your word processing document, and type a descriptive title for your image.

24. Return to the NCBI structure page and find and open the structure "**1OEZ**," [the letter "O" not the number "zero"]. 1OEZ is the structure of the mutant protein.

25. Use the same "**Rendering Shortcuts**" as you did with the reference protein structure.

26. Highlight the mutation location as you did with the reference protein structure.

27. Rotate the protein so that it is oriented in approximately the same way as the reference protein structure image, to see how the mutation affects the shape of the protein.

28. Capture the image of the mutated protein structure, insert it into your document, and type a descriptive title for your screen image.

29. Answer the following questions under your screen images of the reference and mutated protein structures in your document:
    a. In general, how can mutations impact the function of a protein?
    b. How might the specific mutation that you looked at impact the protein?
    c. Answer the following in a few complete sentences: How can tools such as Cn3D help scientists study and treat genetic diseases?

Challenge: Look up the full names of the amino acids involved in the mutation using the "Amino Acid Abbreviations and Chemistry Resources" showing one-letter abbreviations. How are they chemically different? How might this difference impact the protein's function?

Optional: Use your drawing tools in Word® to use an ***arrow*** to point out the location of the mutation in the sequence alignment in the BLAST sequence window and in the Cn3D view. ("**Insert Shapes -> Arrow -> Color**"). If you prefer, you can draw ***red circles*** instead. ("**Insert Shapes -> Oval -> Shape Fill: no fill, Shape outline: red line**"). If you want to crop the image, you can use the ***Crop*** feature in Word®.

PART III: Genetic Testing

Use the information in the background section, as well as your knowledge gained from the genetic testing lessons in this unit, to ***answer the following questions in your word processing document:***

30. How clinically valid is the genetic test? (Low, Medium, or High) Explain why you chose that level.

31. Is there an effective treatment for this condition, whether medical or behavioral? Explain your answer.

32. If you were a genetic counselor, and this condition was found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

33. If you were a genetic counselor, and this condition was not found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

34. What are the characteristics of a good genetic test, in your opinion? To what extent do direct-to-consumer genetic tests that consumers can purchase on their own meet your criteria?

Turn in your Microsoft Word® document in the manner specified by your teacher.

8 Porphyria
Teacher Answer Key

[**Note:** Suggested point values are included for each question, and are intended to provide general guidelines for the weight each question could be given. Using these suggested point values, the total value for this worksheet is ***35 points plus three points for the Challenge questions***.]

PART I: BLAST Protein Alignment

(8 points possible)

7. (+1 point for including a screen capture image of the BLAST alignment with the mutation circled.)

```
>lcl|15815 PATIENT
Length=330

 Score =  678 bits (1750),  Expect = 0.0, Method: Compositional matrix adjust.
 Identities = 329/330 (99%), Positives = 329/330 (99%), Gaps = 0/330 (0%)

Query  1    MQPQSVLHSGYFHPLLRAWQTATTTLNASNLIYPIFVTDVPDDIQPITSLPGVARYGVKR  60
Sbjct  1    ...........L................................................  60

Query  61   LEEMLRPLVEEGLRCVLIFGVPSRVPKDERGSAADSEESPAIEAIHLLRKTFPNLLVACD  120
Sbjct  61   ............................................................  120

Query  121  VCLCPYTSHGHCGLLSENGAFRAEESRQRLAEVALAYAKAGCQVVAPSDMMDGRVEAIKE  180
Sbjct  121  ............................................................  180

Query  181  ALMAHGLGNRVSVMSYSAKFASCFYGPFRDAAKSSPAFGDRRCYQLPPGARGLALRAVDR  240
Sbjct  181  ............................................................  240

Query  241  DVREGADMLMVKPGMPYLDIVREVKDKHPDLPLAVYHVSGEFAMLWHGAQAGAFDLKAAV  300
Sbjct  241  ............................................................  300

Query  301  LEAMTAFRRAGADIIITYYTPQLLQWLKEE  330
Sbjct  301  ..............................  330
```

Figure 1: Screen image of BLAST alignment. Credit: NCBI BLAST.

8. Type a descriptive title for your image.

Example Title: Comparison of Reference and Patient Amino Acid Sequences for ALAD.

(+1 point for including a descriptive title that refers to comparing a patient's sequence and a reference sequence.)

9. Are there any differences between the reference (query) sequence and the patient sequence?

Yes. (+1 point for noting that there is a difference between the two sequences.)

a) Specify where the change is by describing its location by *number* within the protein (for example, at position 181, etc.).

Position 12. (+1 point for correctly noting the position of the change.)

b) Use the one-letter amino acid abbreviations to describe which amino acid has changed, and what the new amino acid is. (For example, M has changed into R).

F has changed to L (F12L).

(+1 point for correctly using the one-letter amino acid abbreviation to note which amino acid (F) was changed to which (L).)

c) Describe whether the change is a substitution (one amino acid exchanged for another), an insertion (an amino acid where there was none before), a deletion, or some other kind of change.

Substitution. (+1 point for correctly noting that the change is a substitution.)

10. Answer the following in a few complete sentences: How can tools such as BLAST help scientists study and treat genetic diseases?

Tools such as BLAST can help compare patient sequences to reference sequences to help determine whether disease-causing mutations are present in the patient.

(+2 points for noting at least two of the underlined phrases above; +1 if students only note that BLAST is used to compare patient sequences to reference sequences without explaining why, i.e., to determine whether mutations are present.)

[**Note:** BLAST can also be used to identify novel mutations associated with disease.]

PART II: Protein Visualization Using Cn3D

(12 points possible plus three possible Challenge points)

19. In one to two sentences, describe where the mutation is located in the protein structure.

The mutation is near where the two protein subunits interact.

(+1 point for noting that the mutation is near where the two subunits interact.)

21. Capture the image of this protein structure with the mutation highlighted, insert it into your word processing document, and type a descriptive title for your image.

Example Title: Location of mutation in reference ALAD protein structure. [A variety of descriptive titles are acceptable.]

(+1 point for inclusion of the image, with the region of the mutation circled (optional), and +1 point for a descriptive title that refers to the mutation and the reference protein structure.)

26. Capture the image of the mutated protein structure, insert it into your document, and type a descriptive title for your screen image.

Example Title: Comparison of mutant (left) and reference (right) ALAD structures. [A variety of descriptive titles are acceptable.]

(+1 point for inclusion of the image, with the region of the mutation circled (optional), and +1 point for a descriptive title that refers to the mutation in the protein structure.)

27. Answer the following questions under your screen image in your document.

a) In general, how can mutations impact the function of a protein?

In general, changes/mutations can alter the protein's shape or chemical characteristics and thus impact the protein's function.

(+1 point for noting that mutations change a protein's shape and/or chemical characteristics and +1 point for noting that this change impacts protein function.)

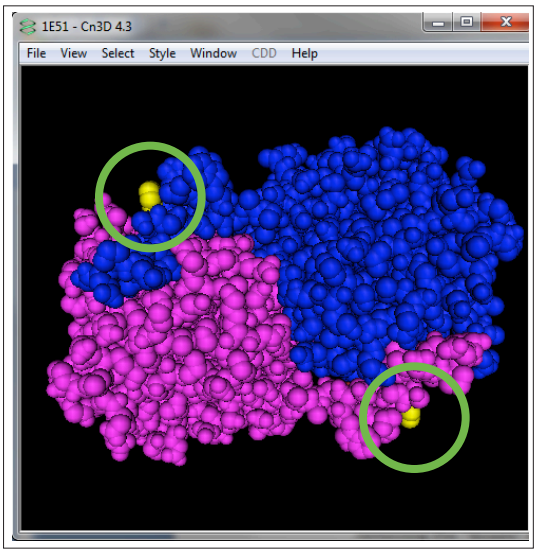

Figure 2: Structure 1E51.
Credit: Adapted from Mills-Davies et al., 2000.

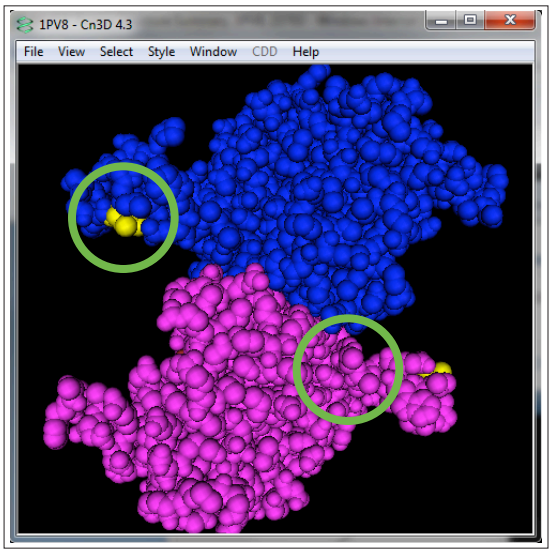

Figure 3: Structure 1PV8.
Credit: Adapted from Brenig et al., 2003.

b) How might the specific mutation that you looked at impact the protein?

> The shape of the protein is drastically altered, with a loop swinging out of the main protein core in the mutant.
>
> (+2 points for a clear explanation of the change in protein shape; +1 point for simply noting that the change in shape impacts protein function.)

c) Answer the following in a few complete sentences: How can tools such as Cn3D help scientists study and treat genetic diseases?

> Tools such as Cn3D can help scientists visualize the location of mutations on important proteins associated with disease. This knowledge can help them understand how the protein's function might be impacted, and the role the protein might play in the development of disease.
>
> (+3 points for inclusion of at least three of the underlined phrases, with an emphasis on demonstration of student understanding that being able to visualize protein shape aids understanding of protein function.)

[**Note:** understanding the structure of a protein associated with a disease-causing mutation might also aid in the development of treatments.]

Challenge: Look up the full names of the amino acids involved in the mutation using the "Amino Acid Abbreviations and Chemistry Resources" showing one-letter abbreviations. How are they chemically different? (1 point) How might this difference impact the protein's function? (2 points) [**Note:** This exercise is considered extra credit.]

> F=Phenylalanine, L=Leucine
> Both are hydrophobic, but phenylalanine is larger, with an [aromatic] ring.
>
> (+1 point for noting that phenylalanine is larger than Leucine; +2 points for explaining that the size difference between the two amino acids can alter the protein's shape and thus impact the protein's function.)

PART III: Genetic Testing

(15 points possible)

28. How clinically valid is the genetic test? (Low, Medium, or High) Explain why you chose that level.

Clinical validity – middle/high (both acceptable). (+1 point) While the test is very reliable, many genes are involved and multiple genes must be tested and completely sequenced in each family.

(+1 point each for noting that the test is reliable and that multiple genes must be sequenced.)

29. Is there an effective treatment for this condition, whether medical or behavioral? Explain your answer.

Yes, effective treatment is available (+1 point), including heme therapy, intravenous glucose, and a high carbohydrate therapy.

(+ 1 point for listing at least one treatment option.)

30. If you were a genetic counselor, and this condition was found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

Recommendations for testing if the condition was found in patient's family: yes, valid test and highly treatable.

(+1 point for recommending 'yes,' +1 point for each reason: valid test available (+1) and effective treatment available (+1).)

31. If you were a genetic counselor, and this condition was not found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

Recommendations for testing if the condition was NOT found in patient's family: no, expensive and labor-intensive. However, if mental illness is a symptom the porphyria test may be informative.

(+1 point for recommending 'no;' +1 for explaining why it is not recommended (because it is expensive and labor intensive); +1 point for explaining the exception in the case of mental illness.)

32. What are the characteristics of a good genetic test, in your opinion? To what extent do direct-to-consumer genetic tests that consumers can purchase on their own meet your criteria?

A good genetic test should be clinically valid (the test accurately predicts a certain clinical outcome, such as getting a particular disease or symptom), and should have an effective treatment, whether through lifestyle modification or clinical invention (surgery, drug treatment, etc.). Many of the tests offered as direct-to-consumer (DTC) genetic tests do not meet these criteria. Some DTC tests have only preliminary scientific research to support their clinical validity, and many have no effective treatment, or treatment options are unclear. In addition, the lack of genetic counseling associated with many DTC tests makes it difficult to advise consumers about these potential shortcomings.

(+2 points for noting good tests are clinically valid (+1) and have effective treatment (+1); +2 points for explaining that many DTC tests do not meet these criteria because: research findings are preliminary (+1), there is no effective treatment (+1), and no genetic counseling is offered to advise patients about the risks.)

8 Amyotrophic Lateral Sclerosis (ALS)
Teacher Answer Key

[**Note:** Suggested point values are included for each question, and are intended to provide general guidelines for the weight each question could be given. Using these suggested point values, the total value for this worksheet is **35 points plus three points for the Challenge questions.**]

PART I: BLAST Protein Alignment

(8 points possible)

7. (+1 point for inclusion of the screen capture image of the BLAST alignment with the mutation circled.)

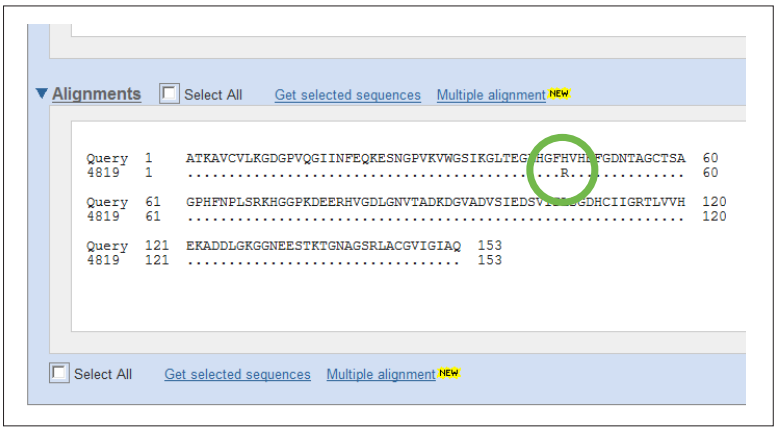

Figure 1: Screen image of BLAST alignment. Credit: NCBI BLAST.

8. Type a descriptive title for your screen shot and answer the following questions under your screen shot in your word processing document.

> Example Title: Comparison of Reference and Patient Amino Acid Sequences for SOD1.
>
> (+1 point for including a descriptive title that refers to comparing a patient's sequence and a reference sequence.)

9. Are there any differences between the reference (query) sequence and the patient sequence?

> Yes. (+1 point for noting that there is a difference between the two sequences.)

a. Specify where the change is by describing its location by *number* within the protein (for example, at position 181, etc.).

> Position 46. (+1 point for correctly noting the position of the change.)

b. Use the one-letter amino acid abbreviations to describe which amino acid has changed, and what the new amino acid is. (For example, M has changed into R).

> H has changed to R (H46R). (+1 point for correctly using the one-letter amino acid abbreviation to note which amino (H) was changed to which (R).)

c. Describe whether the change is a substitution (one amino acid exchanged for another), an insertion (an amino acid where there was none before), a deletion, or some other kind of change.

Substitution. (+1 point for correctly noting that the change is a substitution.)

10. Answer the following in a few complete sentences: How can tools such as BLAST help scientists study and treat genetic diseases?

Tools such as BLAST can help compare patient sequences to reference sequences to help determine whether disease-causing mutations are present in the patient.

(+2 points for noting at least two of the underlined phrases above; +1 if students only note that BLAST is used to compare patient sequences to reference sequences without explaining why, i.e., to determine whether mutations are present.)

[Note: BLAST can also be used to identify novel mutations associated with disease.]

PART II: Protein Visualization Using Cn3D

(12 points possible plus three Challenge points)

17. In one to two sentences, describe where the mutation is located in the protein structure.

The mutation is located within each protein subunit.

(+1 point for noting that the mutation is located within each protein subunit.)

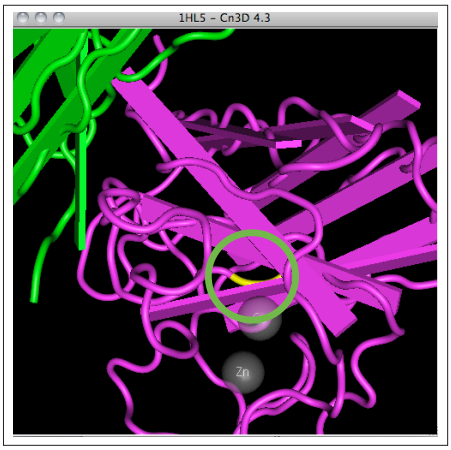

Figure 2: Structure 1HL5.
Credit: Adapted from Strange et al., 2003.

23. Capture the image of this protein structure with the location of the mutation highlighted, insert it into your word processing document, and type a descriptive title for your image.

Example Title: Visualization of location of a mutation in the SOD1 reference protein. [A variety of descriptive titles are acceptable.]

(+1 point for the image and +1 point for inclusion of a descriptive title that refers to the reference protein structure. Note: students may not zoom in this much with their image.)

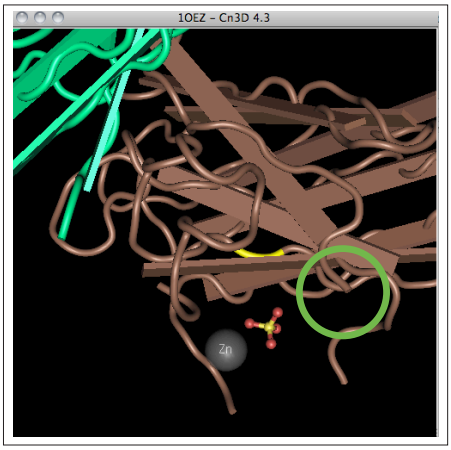

Figure 3: Structure 1OEZ.
Credit: Adapted from Elam et al., 2003.

28. Capture the image of the mutated protein structure, insert it into your document, and type a descriptive title for your screen image.

Example Title: Visualization of location of a mutation in the mutated SOD1 protein. [A variety of descriptive titles are acceptable.]

(+1 point for the image and +1 point for inclusion of a descriptive title that refers to the mutated protein structure.)

29. Answer the following questions under your screen image in your word processing document.

a. In general, how can mutations impact the function of a protein?

> In general, changes/mutations can alter the protein's shape or chemical characteristics and thus impact the protein's function.
>
> (+1 point for noting that mutations change a protein's shape and/or chemical characteristics and +1 point for noting that this change impacts protein function.)

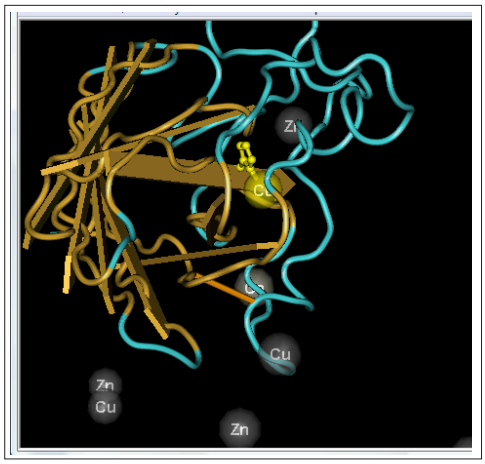

Figure 4: Structure 1OEZ.
Credit: Adapted from Elam et al., 2003.

b. How might the specific mutation that you looked at impact the protein?

> The shape of the protein is drastically altered, with a loop swinging out of the main protein core in the mutant.
>
> (+2 points for a clear explanation of the change in protein shape; +1 point for simply noting that the change in shape impacts protein function.)

c. Answer the following in a few complete sentences: How can tools such as Cn3D help scientists study and treat genetic diseases?

> Tools such as Cn3D can help scientists visualize the location of mutations on important proteins associated with disease. This knowledge can help them understand how the protein's function might be impacted, and the role the protein might play in the development of disease.
>
> (+3 points for inclusion of at least three of the underlined phrases, with an emphasis on demonstration of student understanding that being able to visualize protein shape aids understanding of protein function.)

[**Note:** understanding the structure of a protein associated with a disease-causing mutation might also aid in the development of treatments.]

Challenge: Look up the full names of the amino acids involved in the mutation using the "Amino Acid Abbreviations and Chemistry Resource" showing one-letter abbreviations. How are they chemically different? How might this difference impact the protein's function? [**Note:** This exercise is considered extra credit.]

> H=Histidine, R=Arginine
>
> Both are positively charged, but histidine has an [aromatic] ring, while arginine does not. The histidine has been rendered as a ball and stick (see Lesson Three for a description of how to do this). Students would not be expected to do this during the assessment, but they may remember how to do it.
>
> (+1 point for noting that histidine is larger than arginine; +2 points for explaining that the size difference among the two amino acids can alter the protein's shape and thus impacts the protein's function.)

PART III: Genetic Testing

(15 points possible)

30. How clinically valid is the genetic test? (Low, Medium, or High) Explain why you chose that level.

Clinical validity – middle/low [both acceptable]. (+1 point) Most ALS (90%) is not hereditary, and of the ALS that is inherited, most types (80%) are not due to changes in SOD1. There are no tests currently for those types not due to changes in SOD1. A positive genetic test also does not give information about when symptoms might arise (low penetrance). (+1 point for noting that the test is not reliable or clinically valid, and +1 point for explaining why, i.e., that there are multiple genes involved.)

31. Is there an effective treatment for this condition, whether medical or behavioral? Explain your answer.

Yes, effective treatment is available. (+1 point.) However, currently, there are no preventive treatments, only life-prolonging ones. (+1 point.)

32. If you were a genetic counselor, and this condition was found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

Recommendations for testing if the condition was found in patient's family: no, clinical validity low and even if there is a family history and a negative SOD1 test, there is a chance that the individual might still have a different form of ALS. Also, there are no preventive treatments. However, if an individual strongly desired the test to have as much information as possible to plan for the future, a recommendation could be considered.

(+1 point for recommending 'no,' +1 point for each reason: test is not valid (+1) and no effective preventive treatment available (+1).)

33. If you were a genetic counselor, and this condition was not found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

Recommendations for testing if the condition was NOT found in patient's family: No, even if it is a sporadic case of ALS, the SOD1 test may not be informative for reasons outlined above.

(+1 point for recommending 'no,' +1 point for each reason: test is not valid (+1) and no effective preventive treatment available (+1).)

34. What are the characteristics of a good genetic test, in your opinion? To what extent do direct-to-consumer genetic tests that consumers can purchase on their own meet your criteria?

A good genetic test should be clinically valid (the test accurately predicts a certain clinical outcome, such as getting a particular disease or symptom), and should have an effective treatment, whether through lifestyle modification or clinical invention (surgery, drug treatment, etc.). Many of the tests offered as direct-to-consumer (DTC) genetic tests do not meet these criteria. Some DTC tests have only preliminary scientific research to support their clinical validity, and many have no effective treatment, or treatment options are unclear. In addition, the lack of genetic counseling associated with many DTC tests makes it difficult to advise consumers about these potential shortcomings.

(+2 points for noting good tests are clinically valid (+1) and have effective treatment (+1); +2 points for explaining that many DTC tests do not meet these criteria because: research findings are preliminary (+1), there is no effective treatment (+1), and no genetic counseling is offered to advise patients about the risks.)