Lesson 3: Societal and Biological Perspectives on Alzheimer's Disease

Learning Objectives:

- Students will be able to evaluate what research questions can and cannot be answered via certain datasets based on the demographic composition of the study cohort
- Students will be able to evaluate the known risk factors for Alzheimer's disease
- Students will be able to analyze immunolabeled brain tissue for known biomarkers of Alzheimer's disease pathology
- Students will be able to assess what steps the NIH has taken to increase diversity in biomedical research
- Students will be able to propose possible ways the diversity of biomedical research cohorts can be improved in future research endeavors

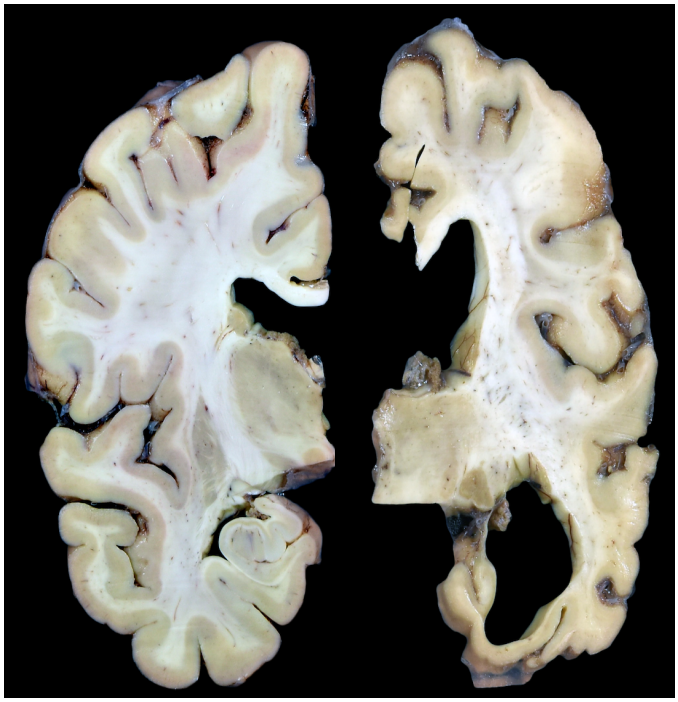


Image from UW Medicine
Left: healthy brain
Right: brain with AD pathology

Introduction

In lesson 1, you learned about the process of brain donation and how biomedical research can use donated brain tissue to perform critical analyses of disease pathology. In this lesson, we will extend our knowledge of brain donation from basic science looking at healthy brains to the basic science of disease. The brain samples donated by these 84 individuals continue to provide crucial data about the early pathogenesis of Alzheimer's disease (AD) for the Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD).

In this lesson, you will use the open data provided by the Allen Institute's SEA-AD study to conduct a qualitative neuropathology image analysis. Prior to looking at the images taken of each donor's brain tissue, you will get to know the demographics of the 84 donors who generously donated their post-mortem brain tissue for the purposes of this study. This lesson consists of three brief activities, which are summarized below:

Activity 1: Biomedical Research and Demographics

- First, you will read a brief article that overviews some history of biomedical research and how it continues to face challenges in recruiting diverse study participants that are representative of the general population.

Activity 2: Exploring the Donor Index

- Next, you will have the opportunity to do a deep dive into the SEA-AD donor index, which provides key demographic information about the 84 donors who were a part of the project.

Activity 3: Neuropathology Image Analysis

- Lastly, you will have the chance to do a side-by-side comparison of images taken of each donor's brain tissue samples and look for specific biomarkers that are suspected to play a role in AD pathology.

At the end of this lesson, you will have developed a deeper understanding of how scientists use brain tissue samples to study the pathology of AD. We hope that you walk away from this lesson recognizing that while an important part of biomedical research is studying **how** a disease impacts people, it is equally important to investigate **who** the disease impacts.

What is Alzheimer's disease (AD)?

AD is a specific type of dementia that affects an individual's behavior, thinking, and memory. AD and dementia are not synonymous:

- **Dementia** is an umbrella term used to describe a class of symptoms that include a decline in memory, reasoning skills, and other cognitive functions. There are several different types of dementia, including vascular dementia, dementia with Lewy bodies, Parkinson's disease dementia, and several others.

- **AD** is a specific disease that accounts for 60-80% of dementia cases. All AD causes dementia, but not all dementia cases are caused by AD.

Since the first known case of AD was documented in the early 1900s, a considerable amount of research has been conducted in an attempt to understand the pathology of the disease. **Pathology** is a term used to describe the general cause and effects of a specific disease.

What is the Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD)?

The SEA-AD project at the Allen Institute for Brain Science is a collective effort from scientists to gain a deep molecular and cellular understanding of the early pathogenesis of AD. The data collected within this study are derived from a full spectrum of 84 older adult donors, also referred to as "aged donors." This cohort of 84 donors includes both healthy controls and those with high AD pathology and cognitive dementia symptoms. In addition to gathering clinical and demographic information from each patient, Allen Institute scientists also gather transcriptomic and pathogenic data from each donor's brain tissue.

Data and specimens were obtained from the Adult Changes in Thought (ACT) Study from Kaiser Permanente Washington Health Research Institute (KPWHRI), and the University of Washington Alzheimer's Disease Research Center (ARDC). The ACT study from Kaiser Permanente specifically follows initially healthy donors starting at 65 years of age and through the rest of their lifespan. This type of longitudinal data allows scientists to gather crucial medical and demographic information about each donor over their lifespan and at their time of death.

The SEA-AD project is one type of basic biomedical research that seeks to understand the early pathogenesis of AD. It is also important to investigate **who** the disease impacts. Historically, biomedical research has struggled to recruit diverse cohorts of study participants. In order to understand why biomedical research has historically studied cohorts of predominately white, cisgender males, we will read the following article by Oh et al. (2015).

Activity 1: Biomedical Research and Demographics

Instructions:

- Read through "Diversity in Clinical and Biomedical Research: A Promise Yet to Be Fulfilled" by Oh et al. (2015) doi: 10.1371/journal.pmed.1001918.
- This is roughly a 10 minute read. The article can be accessed here or by clicking: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4679830/
- After you have finished reading through the article, answer the reflective questions listed below.

Reflective Questions

1. What was the 1993 National Institutes of Health (NIH) Revitalization Act?

2. Since the passage of the NIH Revitalization Act in 1993, what percentage of cancer clinical trials funded by the National Cancer Institute included enough minority participants to meet the NIH's own criteria and goals?

3. What barriers are in place that may discourage some people of color (POC) from participating in biomedical research?

4. What solutions does the article propose that could help to improve the diversity of biomedical research cohorts?

5. What solutions for improving the diversity of biomedical research cohorts can you think of that the article did not mention?

Biomedical Research on Alzheimer's Disease:

As you read in Oh et al.'s 2015 paper, understanding who is impacted by a disease is an integral part of biomedical research. While the field of AD research still has work to do to ensure that it recruits diverse cohorts of study participants, AD researchers have worked hard to identify several possible demographic and/or socioeconomic factors that may have an association with AD. Although there are several suspected demographic factors that impact one's risk for developing AD, this lesson will focus on the following three:

1. Age
2. Race and ethnicity
3. Biological sex

1. **Age:**

Individuals who are 65 or older are at the greatest risk for developing AD. The risk for developing AD doubles every five years after age 65. Age is currently the greatest known risk factor for developing AD.

AD is considered to be younger-onset/early-onset when it occurs in people younger than 65 years of age. Early-onset AD is far less common than AD in people 65 or older. For more information on early-onset AD, please visit:

https://www.alz.org/alzheimers-dementia/what-is-alzheimers/younger-early-onset

2. **Race and ethnicity:**

Both race and ethnicity have been identified as possible risk factors for developing AD. Research has shown that older individuals who identify as Black are roughly twice as likely to develop AD compared to older individuals who identify as White. Additionally, older individuals who identify as Hispanic are roughly 1.5x more likely to develop AD compared to older individuals who identify as White. Research on the racial disparities of AD suggest that the difference in prevalence could be due to socioeconomic factors and not biological factors. These socioeconomic factors could include access to healthcare, access to education, levels of stress experienced by an individual, etc.

Although race and ethnicity are frequently used as synonyms, they carry distinct meanings. The U.S. Census Bureau defines **race** as a person's self-identification with one or more social groups, which can include White, Black or African American, Asian, American Indian, Alaska Native, Native Hawaiian, and/or Other Pacific Islander. Federal statistical standards conceptualize a person's **ethnicity** into one of two categories: Hispanic (or Latino/a/x) or Not Hispanic (Latino/a/x). If a person is Hispanic/Latino, they can self-report/identify as any race.

For more information about race/ethnicity and Alzheimer's disease, please visit:
https://aaic.alz.org/downloads2020/2020_Race_and_Ethnicity_Fact_Sheet.pdf

3. **Biological sex:**

Of the more than 6 million Americans currently living with AD, **roughly two-thirds are female**. Age is the greatest known risk factor for developing AD, and because females tend to live longer than males on average, some scientists believe that the different rates of AD between people of different sexes may be attributable to this difference in average lifespan. However, recent research suggests that the female genome may contain certain **genetic factors** that raise their risk for AD.

Reference:

www.cnn.com/2022/06/30/health/female-alzheimer-gene-discovered-wellness-scn/index.html

Distinguishing between sex and gender:

While sex and gender are frequently used as synonyms, they have distinct meanings. **Sex** is a biological classification that is based on an individual's reproductive organs and sex chromosomes. While XX is typically used as the marker for biological females and XY as the marker for biological males, there are also individuals who are **intersex** and carry other sets of sex chromosomes and/or reproductive organs.

While sex is based on a person's biological characteristics, **gender** is defined as "set of social, psychological, or emotional traits, often influenced by societal expectations that classify an individual as either feminine or masculine." Cisgender individuals identify with the gender they were assigned at birth, while transgender individuals identify as a different gender than assigned at birth. Individuals also can identify as nonbinary or genderfluid. These are by no means the only options for an individual's gender identity, and one's sense of gender identity can be fluid over time.

For more information about gender identity and the difference between sex and gender, visit https://medicine.yale.edu/whr/about/mission/definitions/.

While biological sex and genetics may influence a person's genetic risk for developing AD, a person's **gender** identity may influence the financial or emotional burden that a person experiences due to AD. A majority of AD and dementia caregivers are women, and many women who have taken on caregiving roles for individuals with AD face financial burdens. To learn more about how gender can impact the social and/or financial barriers women face because of AD, please visit https://www.alz.org/alzheimers-dementia/what-is-alzheimers/women-and-alzheimer-s.

The importance of demographics in biomedical research:

Because age, race/ethnicity, and sex have all been identified as possible characteristics associated with AD, it is important that AD studies recruit a diverse range of participants from different age groups, different races/ethnicities, and different sexes. In this next section, you will have the chance to explore the demographic composition of the Allen Institute's SEA-AD donor cohort. Understanding who the donors/study participants are will inform the type of scientific questions we can ask about AD and its pathology.

Activity 2: Exploring the Donor Index

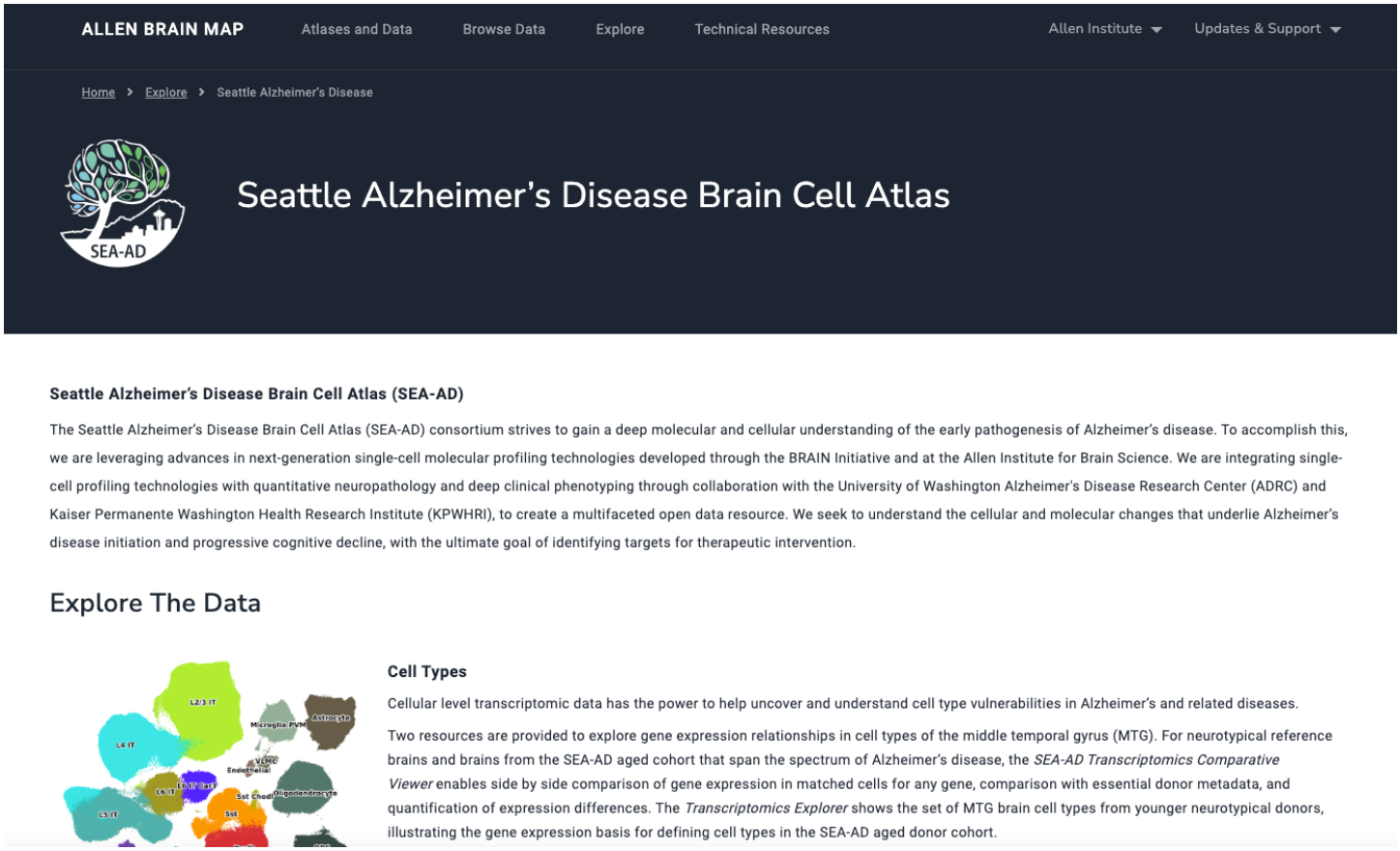
Who are the 84 donors of the SEA-AD study?

In order to explore who the 84 donors of the Allen Institute's SEA-AD project are, we need to first become familiar with how to filter through the data provided in the donor index.

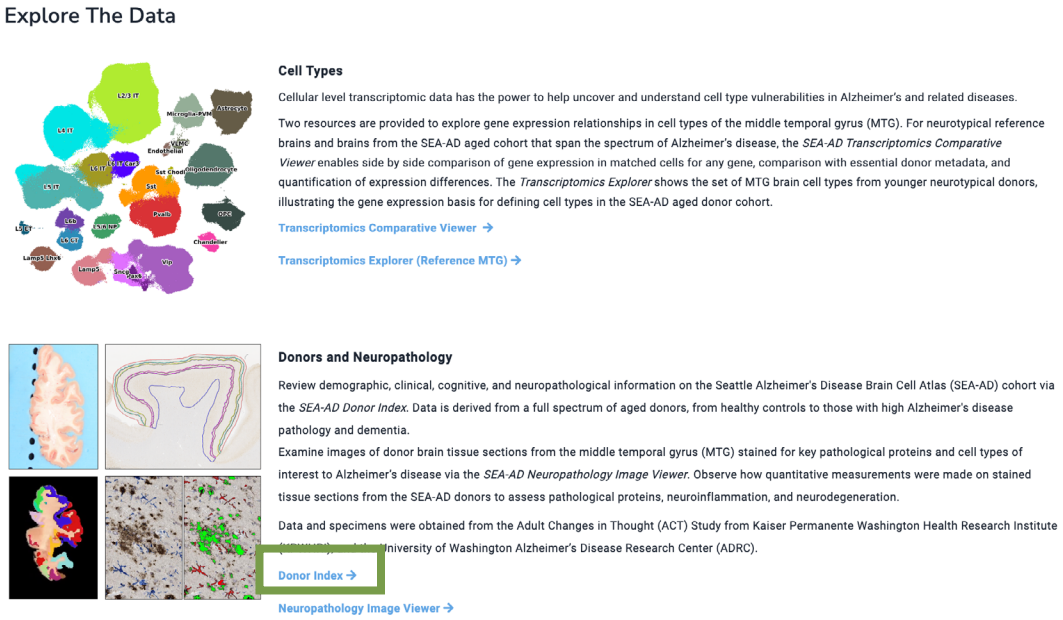
Tutorial: How to Navigate the SEA-AD Donor Index

Step 1: Go to the Seattle Alzheimer's Disease Brain Cell Atlas, linked here:
- https://portal.brain-map.org/explore/seattle-alzheimers-disease

Your screen should look like this:



Step 2: Scroll down this page until you see "Donors and Neuropathology." Under this section, you want to click on "donor index."



After opening the donor index, your screen should look like this:

ALLEN BRAIN MAP | SEA-AD - Metadata and Neuropath | Donors | Terms | Help

Filtered by None

Showing 84 Donors

Donor ID	Age at dea...	Sex	APOE4 sta...	Cognitive s...	Years of ed...	ADNC	Braak stage	Thal phase	CERAD sc...	Lewy body d...	Total micro...
H20.33.040	90+	Male	N	Dementia	13	Intermediate	Braak IV	Thal 4	Frequent	Not Identified ...	0
H20.33.036	90+	Female	N	No dementia	15	High	Braak V	Thal 5	Moderate	Not Identified ...	0
H21.33.019	75	Male	N	No dementia	15	Low	Braak 0	Thal 1	Sparse	Not Identified ...	1
H21.33.040	83	Male	Y	No dementia	17	High	Braak V	Thal 4	Frequent	Olfactory bulb...	3
H20.33.029	90+	Female	N	Dementia	13	High	Braak V	Thal 4	Moderate	Not Identified ...	0
H20.33.015	88	Male	N	Dementia	18	Intermediate	Braak V	Thal 3	Moderate	Not Identified ...	0
H21.33.012	90+	Female	N	Dementia	21	Intermediate	Braak IV	Thal 3	Sparse	Neocortical (D...	0
H21.33.003	78	Male	N	No dementia	16	Not AD	Braak 0	Thal 0	Absent	Not Identified ...	0
H20.33.002	90+	Female	N	No dementia	12	Not AD	Braak IV	Thal 0	Absent	Limbic (Transi...	0
H21.33.030	89	Male	Y	No dementia	17	Intermediate	Braak III	Thal 3	Moderate	Brainstem-pre...	3
H20.33.046	90+	Male	N	Dementia	21	High	Braak VI	Thal 5	Frequent	Not Identified ...	1
H20.33.031	87	Female	N	Dementia	12	High	Braak VI	Thal 4	Frequent	Not Identified ...	1
H20.33.032	90+	Male	N	No dementia	17	High	Braak V	Thal 5	Moderate	Not Identified ...	11
H21.33.027	90+	Male	Y	Dementia	18	High	Braak V	Thal 5	Moderate	Not Identified ...	0

Note in the top left corner, we are told that the data table is currently displaying data for **all 84 donors.**

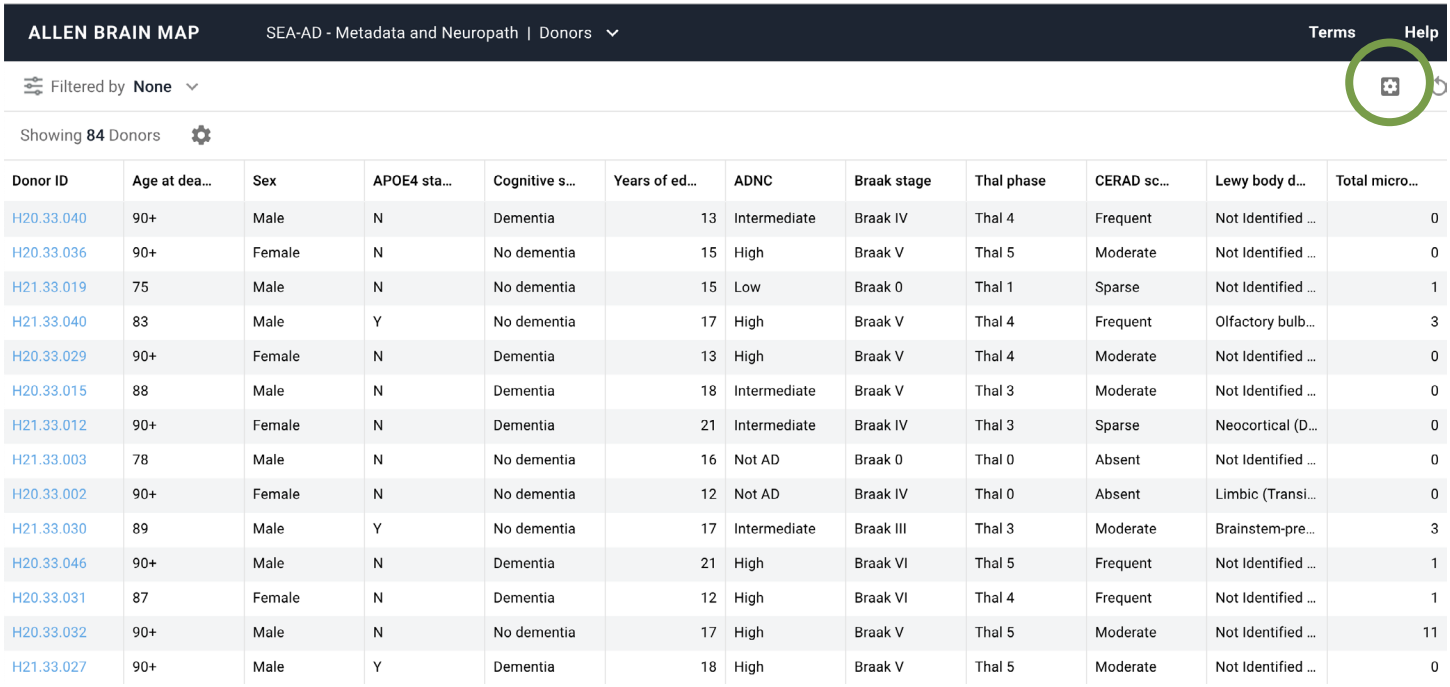
Step 3: Each column contains a different piece of data for each donor. While some are self-explanatory columns, others are less intuitive. Look at the following key for a brief explanation of what each column contains:

Title	Description
Donor ID	Donor Identification Number
Age at death	Age at death
Sex	Biological sex, defined by presence of Y chromosome
APOE4 status	Presence of at least one copy of the apolipoprotein (APOE) e4 allele
Cognitive status	Clinical diagnosis of dementia derived from DSM-IV
ADNC	Overall Alzheimer's disease neuropathologic change score
Thal phase	Extent of the anatomical distribution of amyloid beta plaque deposits
Braak stage	Extent of the anatomical distribution of neurofibrillary tangles (NFTs)
CERAD score	Semiquantitative neuritic plaque density
Lewy body disease pathology	Anatomical distribution of Lewy bodies
LATE-NC stage	Extent of Limbic-predominant Age-related TDP-43 Encephalopathy-Neuropathologic Change
Microinfarcts	Number of microscopic strokes identified in diagnostic screening sections
PMI	Post-mortem interval in hours (time from death to brain removal)
Race/ ethnicity	Self-reported race/ ethnicity
Years of education	Number of years of education starting in grade school
Age of dementia diagnosis	Age when dementia clinically diagnosed
Consensus clinical diagnosis	Clinical diagnosis determined by a consensus of providers who evaluated the individual
CASI score	Most recent Cognitive Abilities Screening Instrument assessment score
Interval from last CASI	Interval (in months) between last CASI assessment and death
MMSE score	Most recent Mini-Mental State Exam assessment score (here derived from CASI components)
Interval from last MMSE	Interval (in months) between last MMSE assessment and death
MoCA score	Most recent Montreal Cognitive Assessment score
Interval from last MoCA	Interval (in months) between last MoCA and death
Fresh brain weight	Brain weight (in grams) at time of brain removal
Brain pH	pH of intraventricular cerebrospinal fluid at time of brain removal
Overall CAA Score	Severity of Cerebral Amyloid Angiopathy
Atherosclerosis	Severity of plaque deposition in arteries
Arteriolosclerosis	Severity of thickening of arterioles
RIN	RNA integrity number of brain tissue sample

Step 4: The default setting is for the donor index to display the data for all 84 donors. However, you can use the filter tools to curate which donors you are evaluating.

There are two options to filter the donor data:

First, go to the top right-hand corner of the spreadsheet and click on the settings icon that looks like a gear. This icon is in the green circle in the figure below:



Donor ID	Age at dea...	Sex	APOE4 sta...	Cognitive s...	Years of ed...	ADNC	Braak stage	Thal phase	CERAD sc...	Lewy body d...	Total micro...
H20.33.040	90+	Male	N	Dementia	13	Intermediate	Braak IV	Thal 4	Frequent	Not Identified ...	0
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H21.33.003	78	Male	N	No dementia	16	Not AD	Braak 0	Thal 0	Absent	Not Identified ...	0
H20.33.002	90+	Female	N	No dementia	12	Not AD	Braak IV	Thal 0	Absent	Limbic (Transi...	0
H21.33.030	89	Male	Y	No dementia	17	Intermediate	Braak III	Thal 3	Moderate	Brainstem-pre...	3
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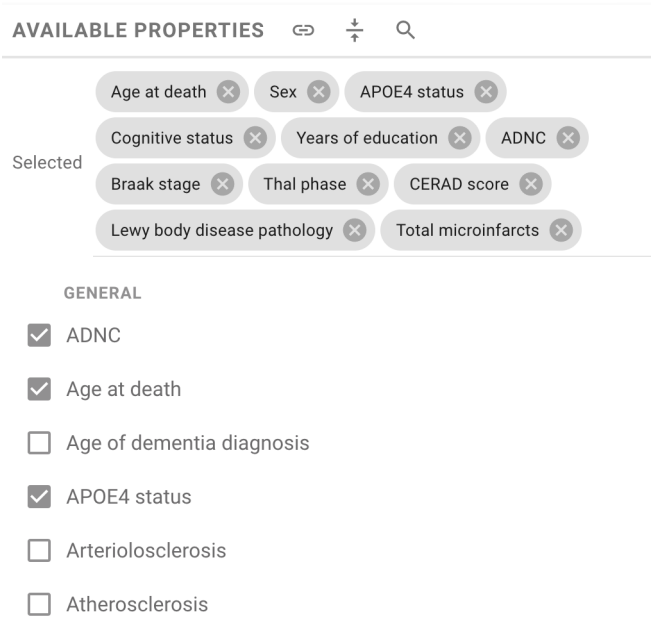
H20.33.032	90+	Male	N	No dementia	17	High	Braak V	Thal 5	Moderate	Not Identified ...	11
H21.33.027	90+	Male	Y	Dementia	18	High	Braak V	Thal 5	Moderate	Not Identified ...	0

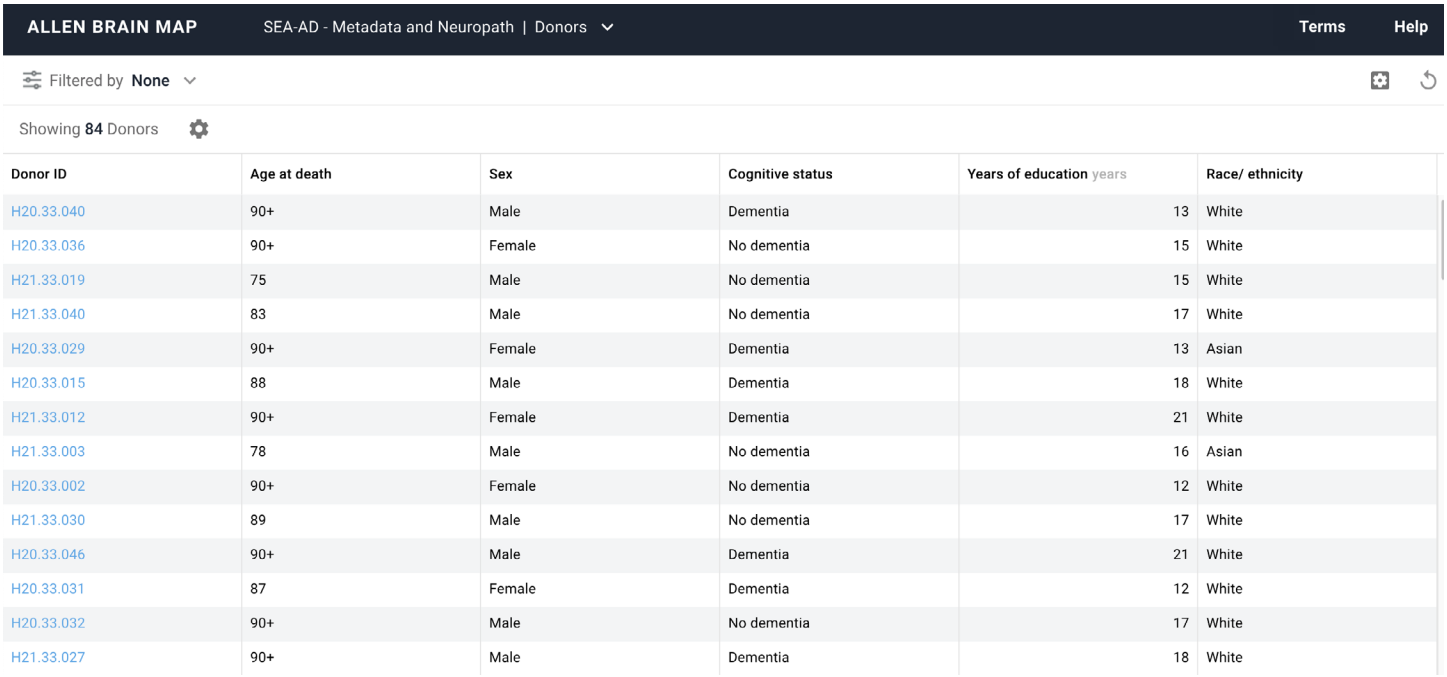
A drop-down menu should display in the top right corner of your screen.

Step 5: Using this drop-down menu, select the following criteria to filter the data by (note: you will also have de-select some of the characteristics that the donor index selects by default):

- Age at death
- Sex
- Cognitive status
- Years of education
- Race/ethnicity

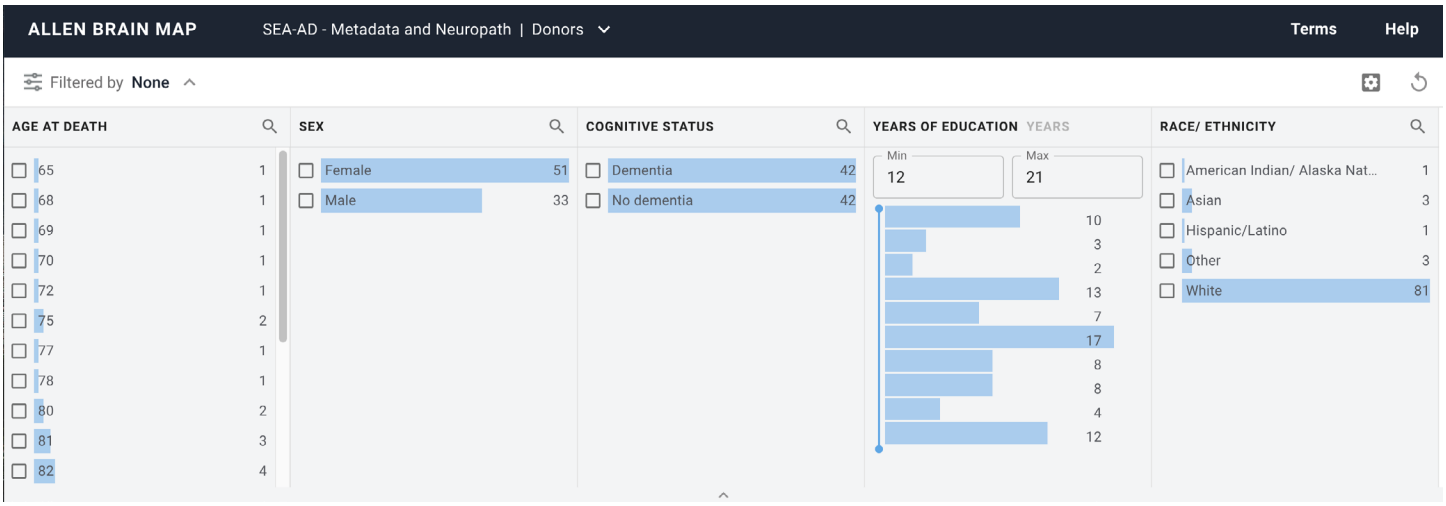


Notice that selecting these criteria in the “available properties” drop-down menu changes the table so that it looks like this:



Donor ID	Age at death	Sex	Cognitive status	Years of education years	Race/ ethnicity
H20.33.040	90+	Male	Dementia	13	White
H20.33.036	90+	Female	No dementia	15	White
H21.33.019	75	Male	No dementia	15	White
H21.33.040	83	Male	No dementia	17	White
H20.33.029	90+	Female	Dementia	13	Asian
H20.33.015	88	Male	Dementia	18	White
H21.33.012	90+	Female	Dementia	21	White
H21.33.003	78	Male	No dementia	16	Asian
H20.33.002	90+	Female	No dementia	12	White
H21.33.030	89	Male	No dementia	17	White
H20.33.046	90+	Male	Dementia	21	White
H20.33.031	87	Female	Dementia	12	White
H20.33.032	90+	Male	No dementia	17	White
H21.33.027	90+	Male	Dementia	18	White

Step 6: In addition to the “available properties” drop-down menu, there is also a way to directly filter the data by clicking on the arrow next to “Filtered by: None.” After clicking on the arrow, your screen should look like this:



Notice that the options to filter the data match the one’s you selected in the “available properties” box. The blue bars help to visualize the extent to which the donors fall into the selected categories. The numbers next to each trait tell you the exact number of donors that fall into each of the categories.

Knowledge Check

Now that you know how to filter the data, let's think back to the three demographic features we learned about earlier that possibly play a role in risk for developing AD:

1. Age
2. Race/ethnicity
3. Sex

1. Would this cohort of 84 donors allow us to study the possible association between race/ethnicity and AD? Why or why not?

2. Would this cohort of 84 donors allow us to study the possible association between sex and AD? Why or why not?

Understanding the strengths and limitations of your data:

The lack of racial and ethnic diversity of the 84 donors in this study is one of its limitations. Earlier in the lesson, you read Oh et al.'s (2015) article on the lack of diversity in biomedical research studies. The field of science continues to make efforts towards addressing this demographic gap in its research to ensure that our study populations are representative of society as a whole. Conclusions from this study with regard to race and ethnicity are limited, but we can explore impacts with regard to sex and gender. While everyone is encouraged to donate their brains to science, it is imperative that people feel comfortable doing so. Improving outreach and education efforts to diverse audiences would help the field of biomedical sciences deepen its foundational knowledge of AD pathology.

While this cohort of 84 donors does not allow us to explore questions about a possible association between AD and race/ethnicity, this cohort does allow us to ask several interesting and crucial questions about a possible association between a person's sex and their risk for developing AD.

In order to explore the possible association between sex and AD, we can use the collection of neuropathology image data available from the Allen Institute's SEA-AD study.

Activity 3: Neuropathology Image Analysis

As a part of the SEA-AD study, Allen Institute scientists imaged sections of donor brain tissue from the middle temporal gyrus (MTG) of all 84 donors. Before imaging these samples, the scientists applied a variety of stains that dye specific proteins and cell types of interest that are believed to play a role in AD pathology. But how do the scientists know what to look for in the brain tissue?

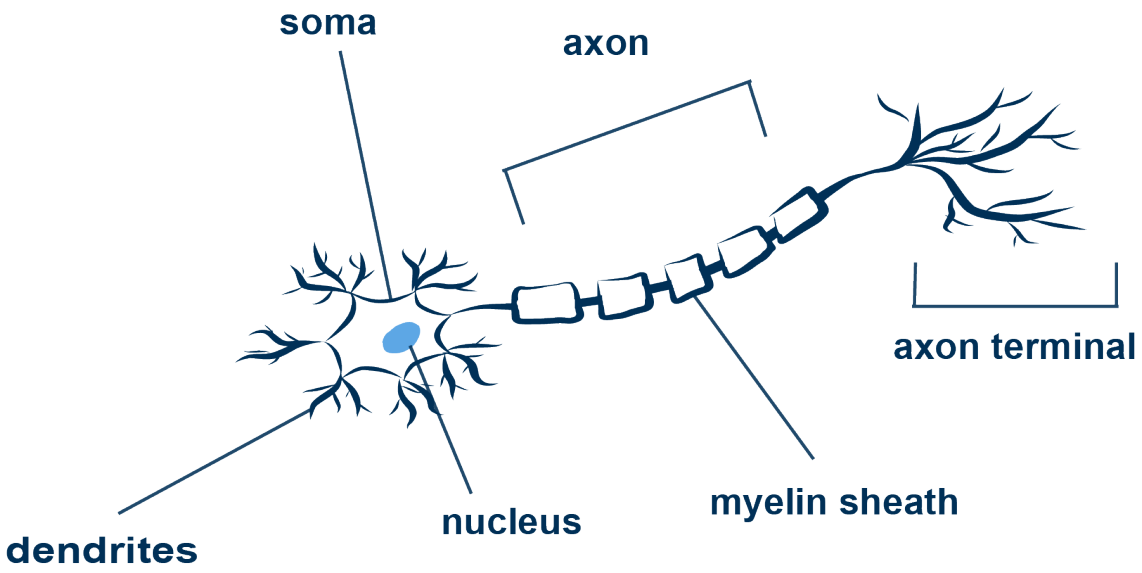
In order to look for pathological signs of AD in each donor's sample, scientists look for specific biomarkers. **Biomarkers** are biological signs of disease. Research on AD has identified several possible biomarkers for the disease. While several different possible biomarkers have been identified by AD researchers, this lesson will focus on two in particular: **beta-amyloid plaques** and **neurofibrillary (tau) tangles**.

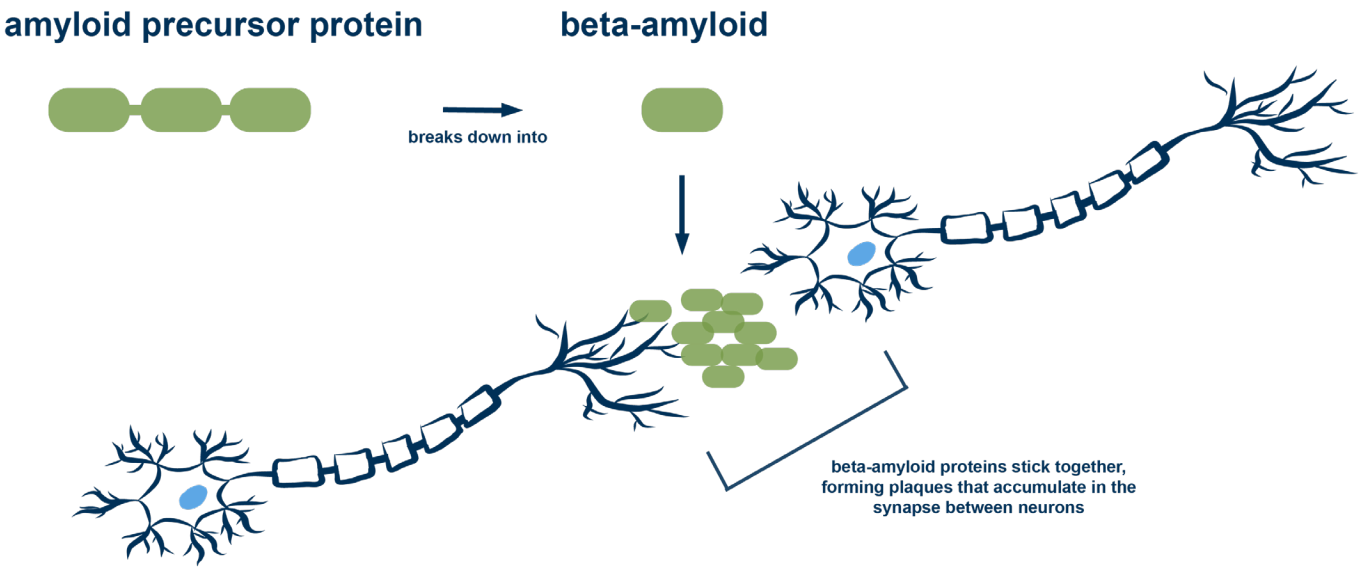
Before looking at the images using the Allen Institute's SEA-AD Neuropathology Image Viewer, we will explore what beta-amyloid plaques and neurofibrillary (tau) tangles are and the current theories surrounding how they play a potential role in AD pathology.

Note: The images below depict cartoon diagrams of neurons and non-neuronal cells. While these cartoons can be helpful to understand basic neural anatomy, they do not capture the full complexity and/or diversity of brain cells. For a deep dive into the structure and function of neurons, please check out the Neurons: Beyond the Textbook lesson located at https://alleninstitute.org/about/education-outreach/neurons-beyond-textbook/.

1. Beta-Amyloid Plaques

Research into AD biomarkers has identified beta-amyloid plaques as a possible biological hallmark of AD pathology. Amyloid precursor protein (APP) is a larger protein that is broken down into a smaller protein called beta-amyloid 42. In patients with AD, abnormally high levels of beta-amyloid appear to clump together to form plaques. These plaques can accumulate in the synapses between neurons and disrupt neuronal functioning.





Scientists measure the extent of the anatomical distribution of beta-amyloid plaque deposits by organizing pathology into different Thal phases. The **Thal phases** range from stage 0 (least severe extent of beta-amyloid plaque accumulation) to stage 5 (most severe beta-amyloid plaque accumulation).

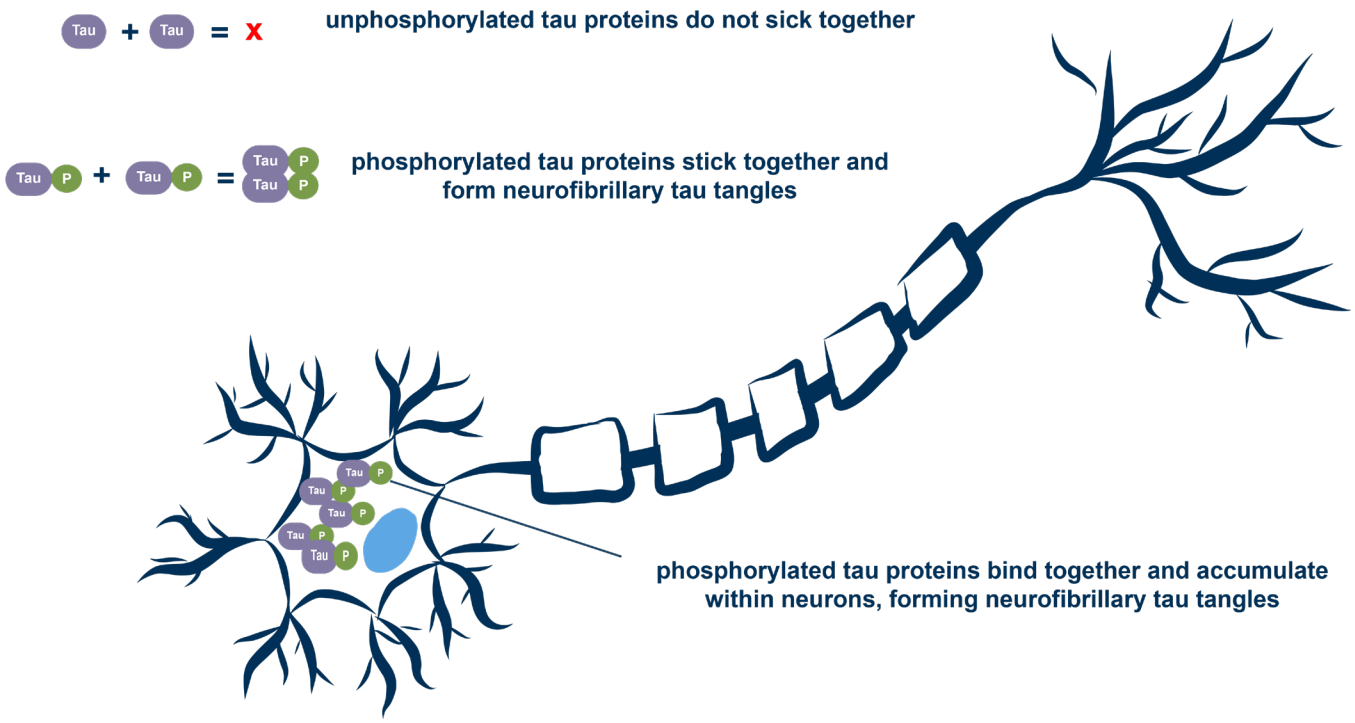
Reference: https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease

2. Neurofibrillary (Tau) Tangles:

An additional potential biomarker for AD is neurofibrillary (tau) tangles. While beta-amyloid plaques accumulate in between neurons, these tau tangles appear to accumulate at abnormally high levels inside neurons. Tau proteins in healthy neurons typically bind to microtubules. In patients with AD, these tau proteins undergo a chemical change when they are phosphorylated. This phosphorylation causes tau proteins to bind to each other rather than to microtubules, and these tau "tangles" then accumulate inside neurons. The accumulation of these tangles appears to disrupt the transport system within a neuron.

Scientists measure the extent of the anatomical distribution of neurofibrillary tangles by organizing pathologies into Braak stages. **Braak stages** range from stage 0 (least severe amount of neurofibrillary tangles) to stage 6 (most severe extent of neurofibrillary tangles). Roman numerals are frequently used to describe Braak stages. See below for how to interpret roman numerals for numbers 1-6:

- 1 = I
- 2 = II
- 3 = III
- 4 = IV
- 5 = V
- 6 = VI



Neuropathology Image Analysis:

Now that you are familiar with a few of the suspected biomarkers for AD, you can use this knowledge to look at actual neuropathology images from the donors in the Allen Institute's SEA-AD study. In particular, we will be looking at the presence/absence of beta-amyloid plaques within each tissue sample, as well as the relative distribution of these plaques between patient samples.

The neuropathology image viewer organizes the images by each donor's ID number. The Allen Institute uses a donor ID number rather than the donor's name in order to maintain the donors' anonymity and to respect their privacy.

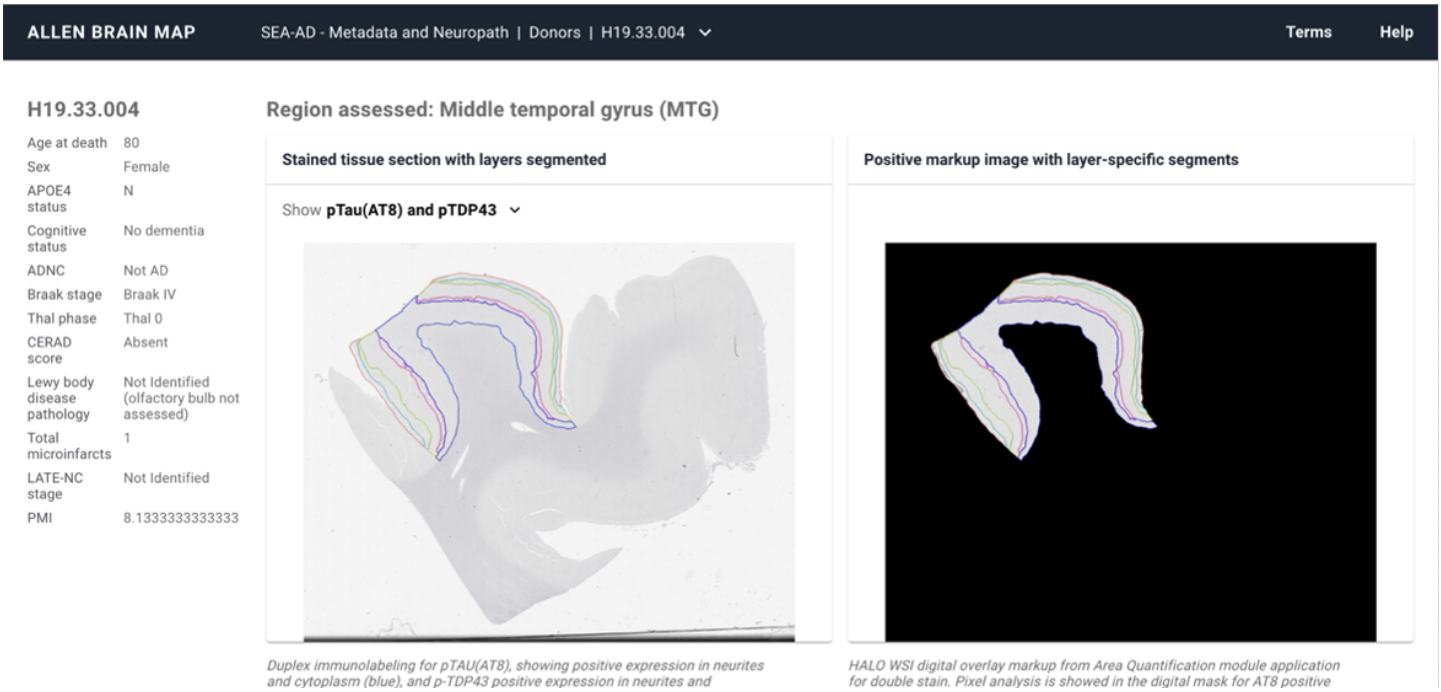
Donor H19.33.004:

We still start by looking at the neuropathology images of donor H19.33.004.

Step 1: Open up the neuropathology image viewer for donor H19.33.004:

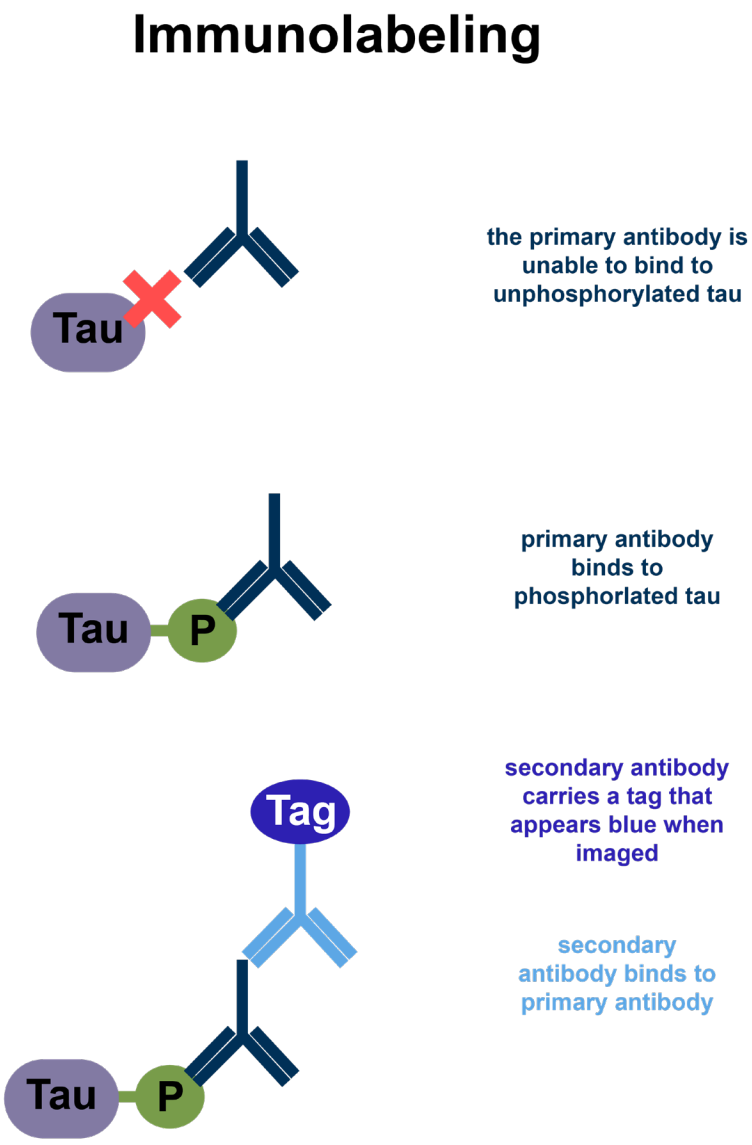
https://knowledge.brain-map.org/data/JGN327NUXRZSHEV88TN/donors/B2YX5RFBGNHG-F6R18GG

Your screen should look like this:

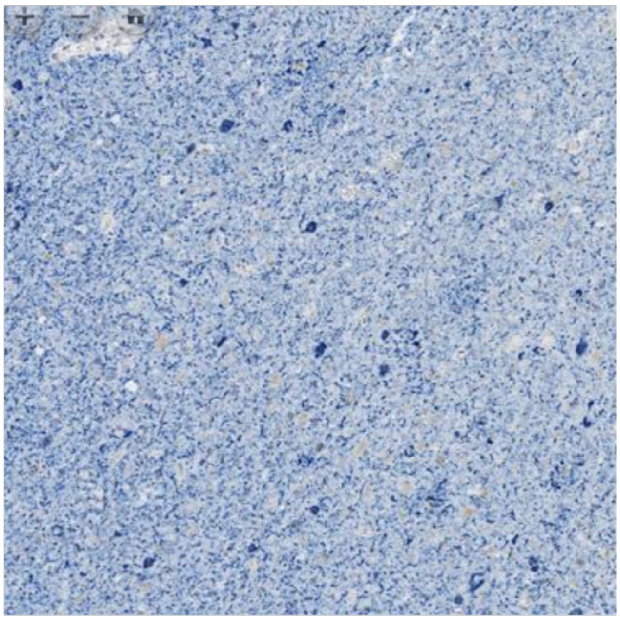


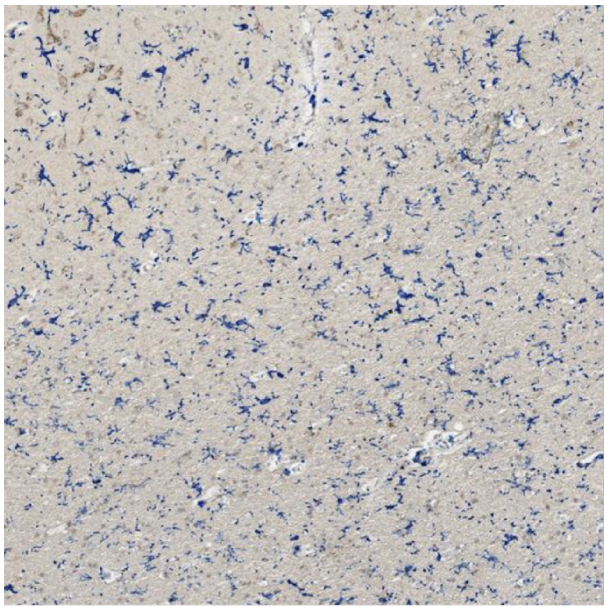
There are two different options for viewing this tissue sample. On the left, you can view the image under the "stained tissue section with layers segmented" option. The lefthand panel allows you to choose which immunolabeled tissue sample you are looking at.

Immunolabeling is the process where antibodies are used to visualize specific proteins. See the figure below to see the process of immunolabeling:



- **Which of the following images of immunolabeled brain tissue appears to have a larger amount of phosphorylated tau protein (tau tangles)? How do you know?**

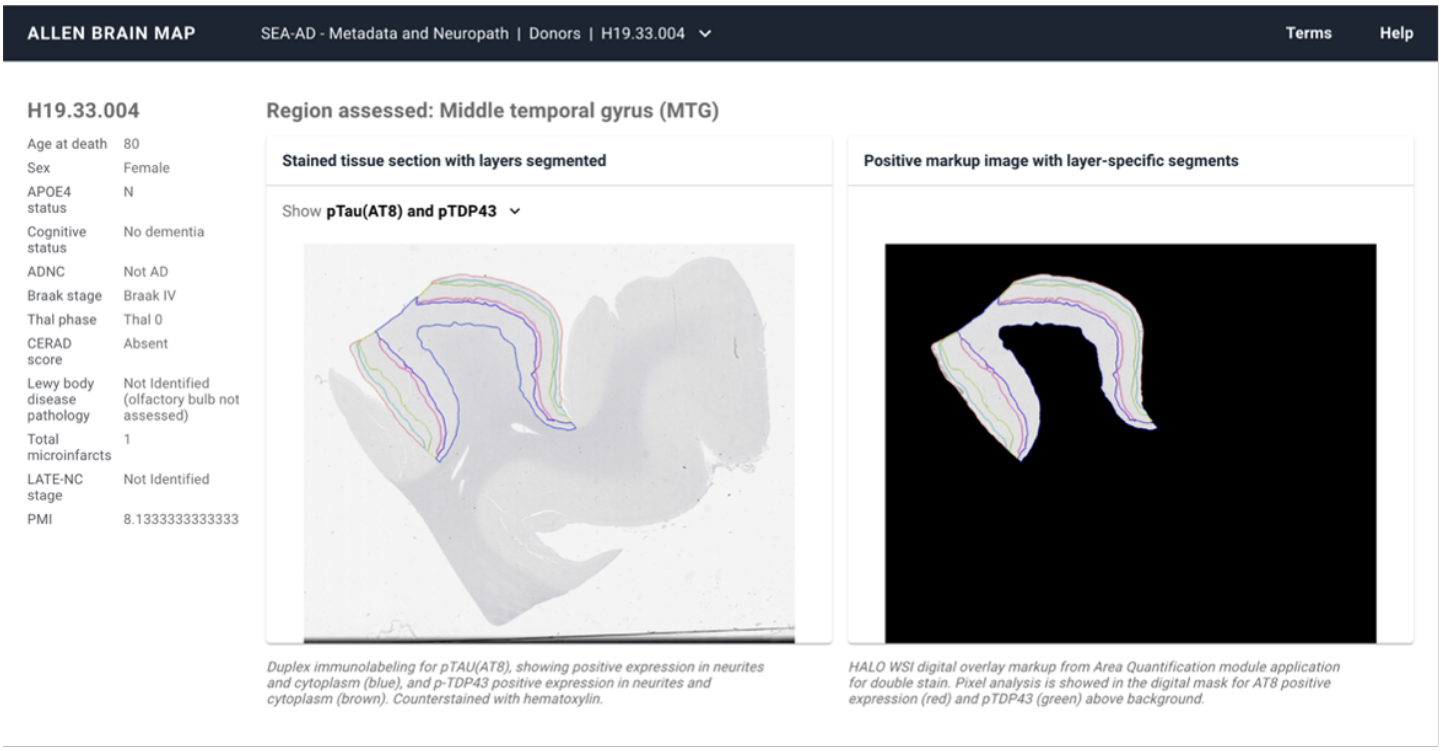




Please write your answer in the box below:

The default setting shows immunolabeling for "**Abeta(6E10) and IBA1**," which allows researchers to stain for beta-amyloid plaques. You can change this setting to select for stains for a variety of proteins. We will be changing the immunolabeling we are looking at in order to search for the presence or absence of neurofibrillary (tau) tangles.

Step 2: Click on the drop down menu and change the immunolabel from "Abeta(6E10) and IBA1" to "**pTau(AT8) and pTDP43**." Your screen should now look like this:



The **pTau(AT8)** and **pTDP43** are two different immunolabels that allow researchers to visualize the following proteins in tissue samples:

• **pTau(AT8):** AT8 stands for anti-phospho-tau and is a monoclonal antibody that binds to **phosphorylated tau protein**. Remember from before that phosphorylated tau protein tends to form neurofibrillary tangles that can accumulate within neurons. These tangles are suspected to play a role in AD pathology, and thus are often viewed as hallmark of AD.

• **pTDP43**: This stands for phosphorylated transactive response DNA-binding protein 43 (TDP43). This protein is suspected to form pathologic aggregates in AD
Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3830649/

Recall from the immunolabeling process that the secondary antibody used is connected to a fluorescent tag that allows scientists to localize and visualize the proteins of interest. Allen Institute scientists used a tag for **phosphorylated tau tangles** that appears blue and a tag for **phosphorylated TDP43** that appears brown.

On the right, the "positive markup image with layer-specific segments" displays only part of the sample that has been labeled by layer of the cortex. In this image, phosphorylated tau proteins (tangles) appear red and phosphorylated TDP43 protein appears green. The colored lines on the image in blue, pink, green, and yellow are separating out the different layers of the cortex.

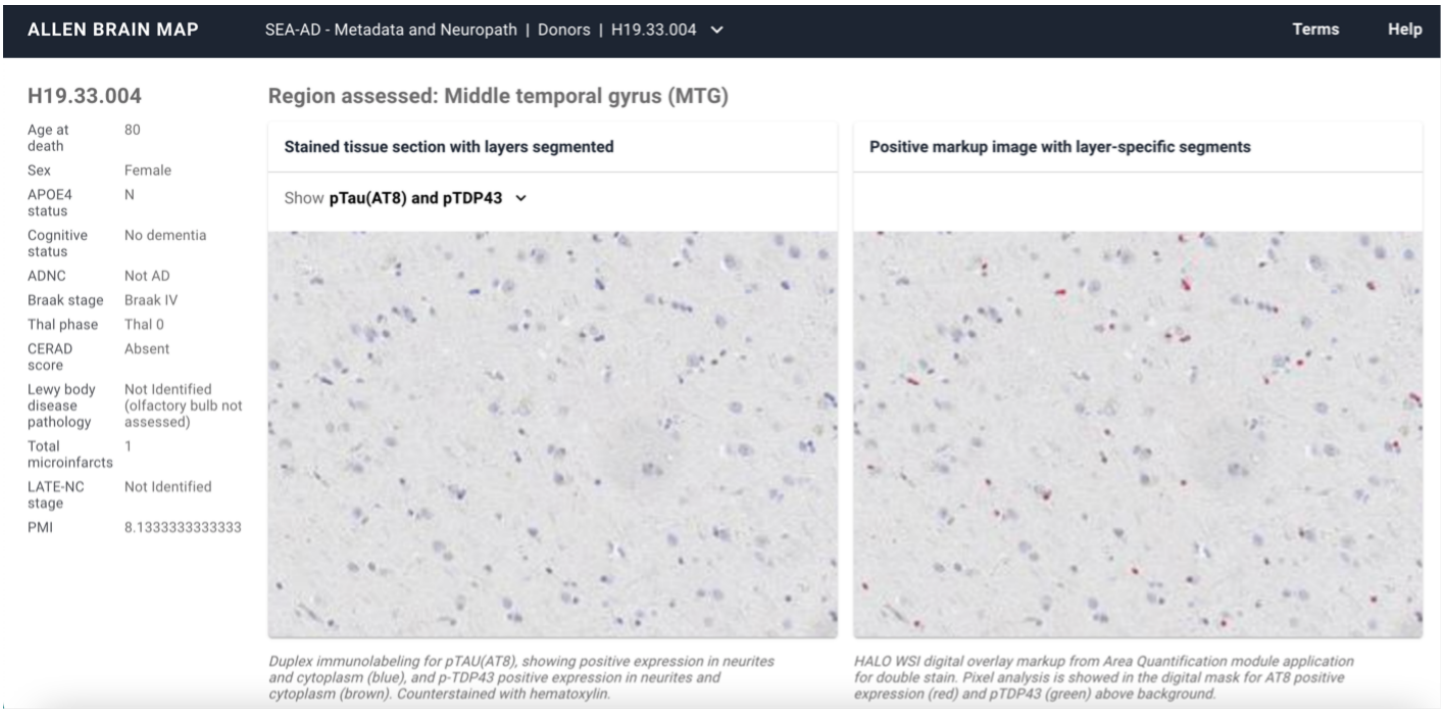
To get acquainted with the neuropathology image viewer, fill in the following table about donor H19.33.004:

	Donor H19.33.004
Sex	
Cognitive Status	
ADNC	
Braak Stage	

1. What does ADNC tell us about the donor? (Hint: if you are unfamiliar with what ADNC measures, please look back at the key featured earlier in the lesson, which is also linked here.)

2. What does the Braak stage tell us about the donor?

Look at the image on the left. Zoom in to any part of the sample. This is one example of what you see, but your image could look different based on where you zoom in:



1. Do you see any phosphorylated tau tangles in this sample?

2. Based on the characteristics of donor H19.33.004, did you expect to see a large or small number of phosphorylated tau tangles? Explain your answer.

Donor H20.33.031:

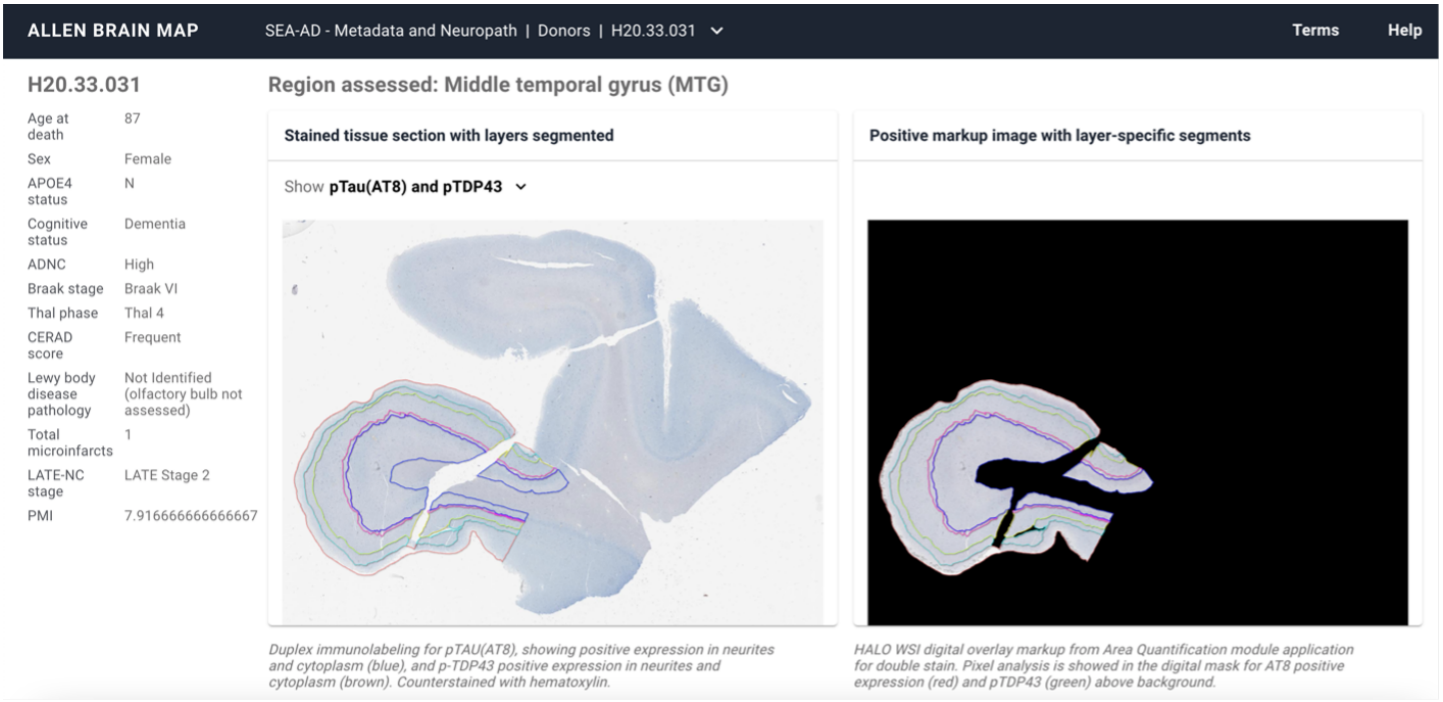
The neuropathology image viewer allows us to compare images between donors. In a **new tab**, go to:

https://knowledge.brain-map.org/data/JGN327NUXRZSHEV88TN/donors/A1NYU4F5DY621E8HO43

Note: opening this link in a new tab will allow you to do a side by side comparison of donor H19.33.004 from before and H20.33.031.

Change the immunolabel to **pTau(AT8) and pTDP43**.

Your screen should look like this:



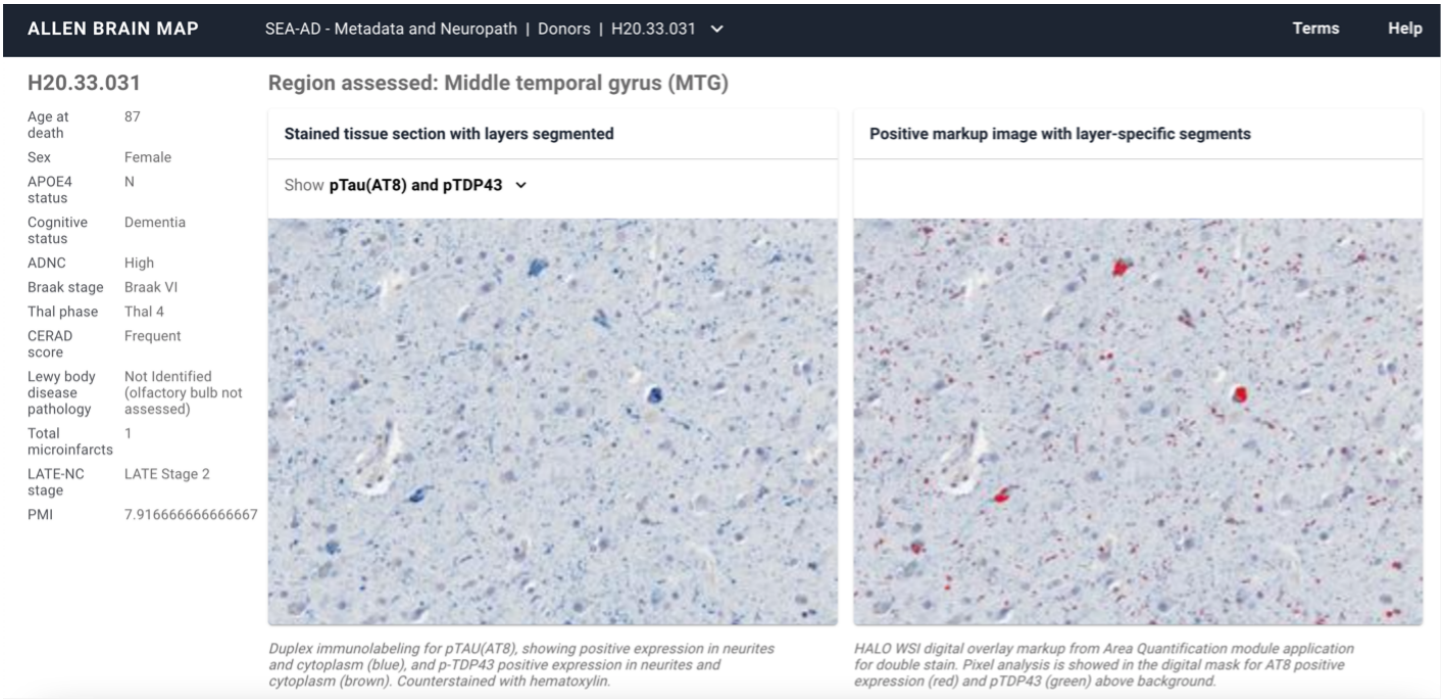
On the following page, fill in the table below to orient yourself to the characteristics of this donor:

	Donor H20.33.031
Sex	
Cognitive Status	
ADNC	
Braak Stage	

1.Notice that the stained tissue section with layers segmented (the image on the left) has a different overall shape than the tissue section from the previous donor (H19.33.004). Why do you think that is?

2. Looking only at the donor characteristics, what is different between donor H19.33.004 and H20.33.031?

Zoom in on the image on either the left or the right. This is one example of what you might see, although the image could differ based on where you chose to zoom in:



1. Do you see any tau tangles in this image?

2. Based on the characteristics of donor H19.33.004, did you expect to see a large or small number of tau tangles? Explain your answer.

3. Looking only at the neuropathology images, what is different between donor H19.33.004 and H20.33.031?

After comparing the neuropathology images between two female donors, let's do a comparison amongst male donors.

Donor H21.33.019 and Donor H20.33.046:

In a new tab, open: https://knowledge.brain-map.org/data/JGN327NUXRZSHEV88TN/donors/6JRB7FMYBVE5F3HDTM5

In ANOTHER new tab, open: https://knowledge.brain-map.org/data/JGN327NUXRZSHEV88TN/donors/HB9ENTM30SNBC4EP0PU

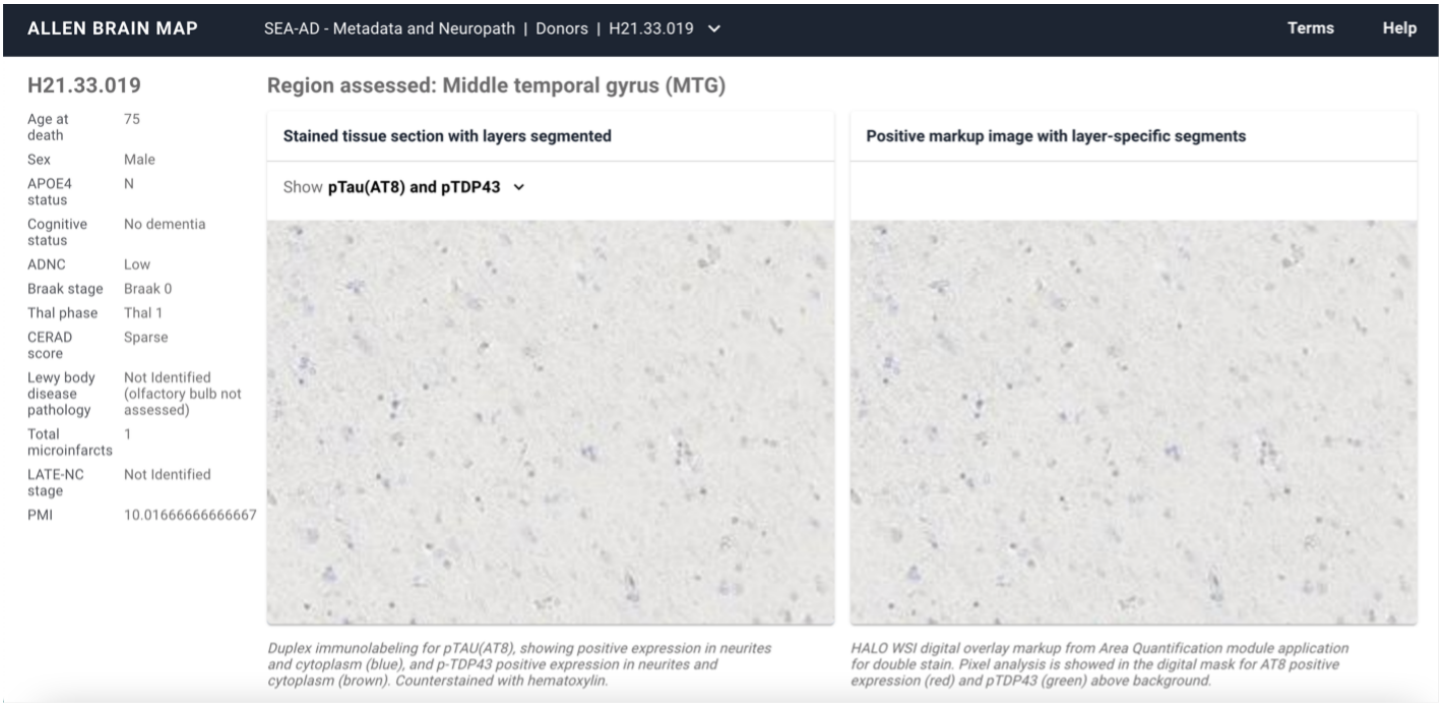
To clarify: in one tab you should have open the neuropathology images for donor H21.33.019, and in another tab you should have open the images for donor H20.33.046.

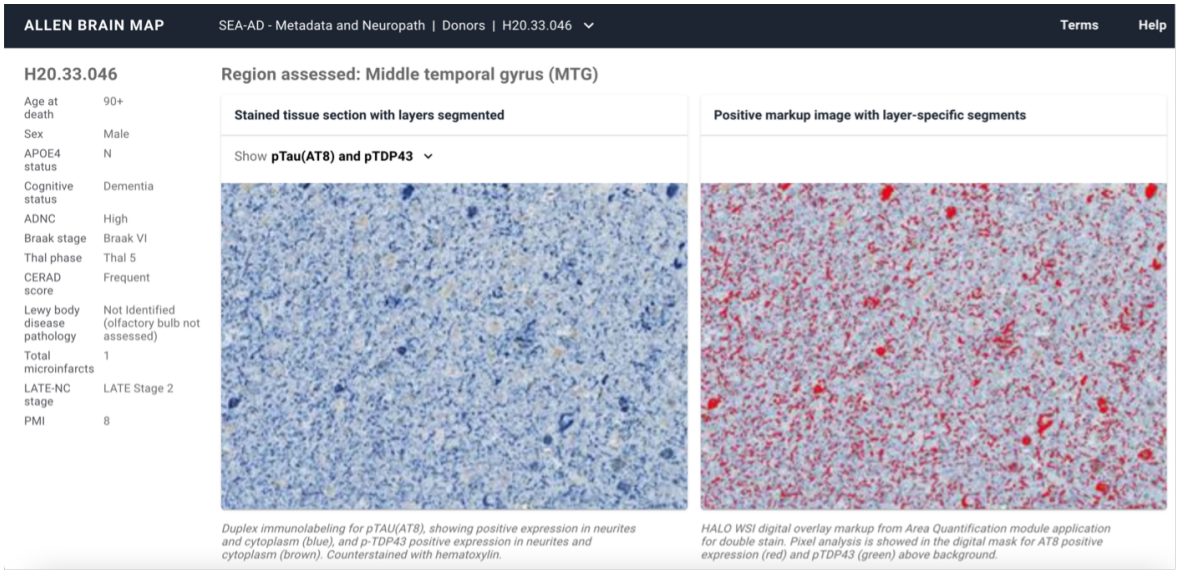
For both of these donors, be sure to change the immunolabel from the default to the pTau(AT8) and pTDP43 immunolabel.

Fill in the table below to do a side-by-side comparison of the two donors and their characteristics:

	Donor H21.33.019	Donor H.20.33.046
Sex		
Cognitive Status		
ADNC		
Braak Stage		

Zoom in on both of the donor images to compare:





1. What do you notice is similar between the donor images?

2. What do you notice is different between the donor images?

3. Did you expect donor H.21.33.019 and donor H.20.33.046 to have similar neuropathological images or different? Why or why not?

Qualitative vs. Quantitative Analyses:

Qualitative Analysis:

The comparative analysis you just performed was a qualitative assessment of Alzheimer's neuropathology. We were looking for the presence/absence of beta-amyloid plaques, but we were not attempting to quantify or measure the amount of these plaques.

Quantitative Analysis:

After scientists perform a qualitative analysis of neuropathology images, they often continue their analysis by quantitatively measuring the amount of biomarkers present in a sample.

Reflective Questions

1. After viewing the neuropathology images, how would you predict scientists go about quantitatively measuring biomarkers within a sample?

2. A common next step for scientists in a quantitative image analysis is to perform an expert annotation of the sample. What do you think it means for someone to expertly annotate a neuropathology image?

3. After looking at these neuropathology images, what other things would you want to explore in future qualitative or quantitative analyses of these images?

Conclusion:

Throughout the course of this lesson, you had the opportunity to explore AD pathology through qualitatively analyzing neuropathology images from the Allen Institute for Brain Sciences' SEA-AD project. We began the lesson by reflecting on the history of biomedical research and how the field of science continues to strive toward improving the diversity of its study cohorts. After exploring the demographic characteristics of the 84 donors included within the Allen Institute's SEA-AD study, you viewed the neuropathology images from a few of these donors.

In lesson 4, you will have the opportunity to explore AD pathology even further by analyzing transcriptomic data. Rather than view specific images of the brain tissue, you will instead analyze transcriptomic data from each donor's brain sample and explore how scientists can use data about gene expression to explore AD pathology.

For more information on the Allen Institute for Brain Science's SEA-AD project, please visit: https://portal.brain-map.org/explore/seattle-alzheimers-disease.

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