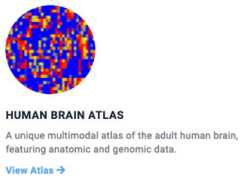
Supplementary Material for Ryan J, Casimo K (2021) A Course-Based Research Experience Using the Allen Brain Map: From Research Question to Poster Session. J Undergrad Neurosci Educ 19(2):A260-A266.

NEUROBIOLOGY 340

TUTORIAL 3: THE HUMAN BRAIN ATLAS

Throughout this tutorial, you will be directed to answer questions about what you find. These questions can be found on the last page of the tutorial.

Go to http://portal.brain-map.org and select Allen Human Brain Atlas from the options available. You can use the drop-down menu at the top of the page or search through the icons for this one:



The Allen Human Brain Atlas offers different types of searches to allow a user to:

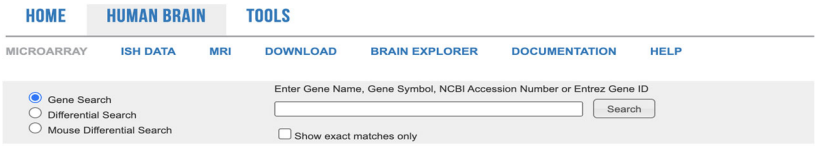
(1) Obtain gene expression data for specific genes (or probes) of interest (Gene Search)

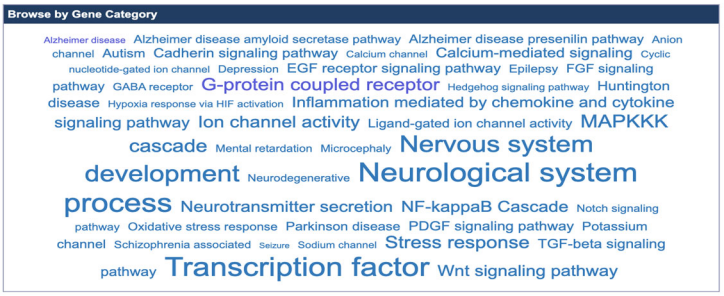
(1a) Obtain gene expression data for many related genes (Browse by Gene Category)

(2) Compare expression between different anatomic regions (Differential Search)

(3) Use a 'seed' gene to find other genes with similar expression patterns (Find Correlates - access via Gene Search)

You can also search in comparison to the Allen Mouse Brain Atlas (Mouse Differential Search), which we will not use in this tutorial.





Let us start with the gene search.

Part I: Gene Search

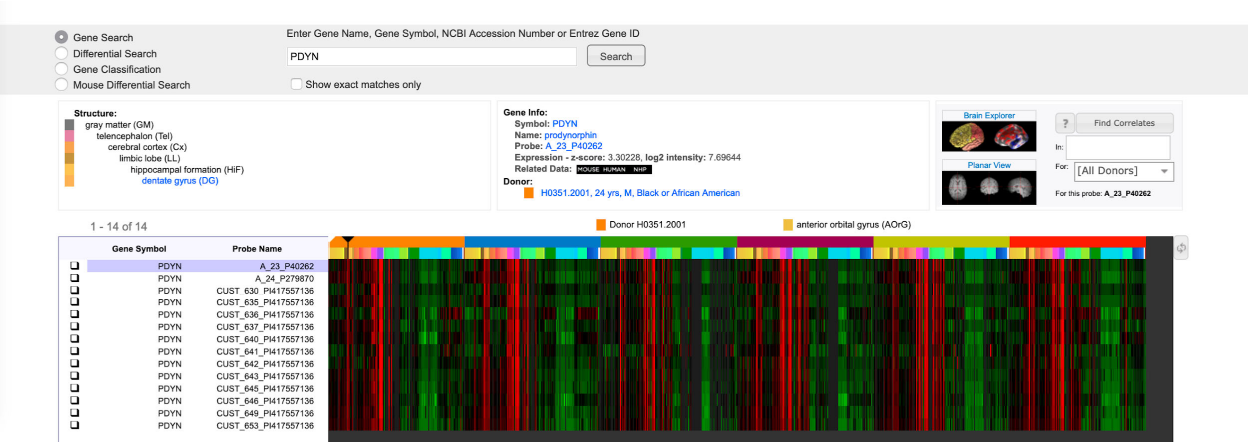
Let us search for gene PDYN in the Allen Human Brain Atlas to learn where it is expressed, and then learn what it does.

This atlas uses a method called microarray to measure gene expression. In this method, molecular probes are attached to a chip (the microarray) and extracts from each brain region are applied. The RNA corresponding to each gene attaches itself to the probe, letting us estimate the relative amount of RNA for that gene in each region.

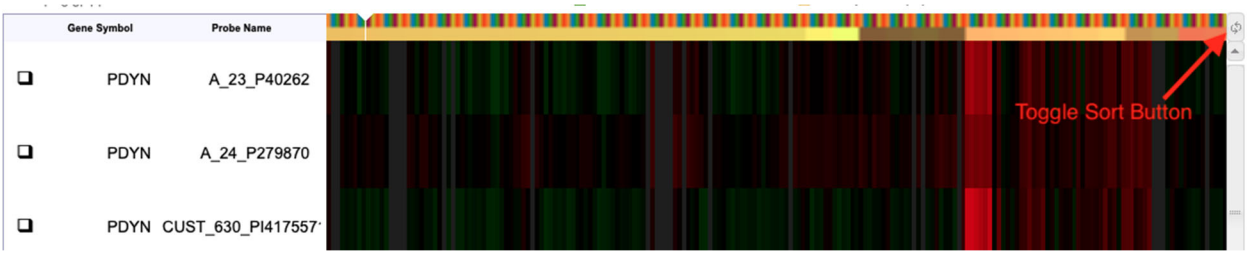
Go to NCBI at https://www.ncbi.nlm.nih.gov/gene/ and do a gene search to understand what PDYN does. *Answer Q1 on the last page of this tutorial*.

Enter PDYN in the search box and make sure the radio button is set to *Gene Search.* To show exact matches to your search query click the "*Show exact matches only*" box. There are multiple probes for this gene, which attach to different stretches of the RNA. You can see the results are all very similar - just use the first probe for this tutorial.

This search returned a heatmap for the gene you specified. At the top of the heatmap are two colored bars. The top bar above the heat map sorts expression by the donor and lower bar by brain structure. *Answer Q2*.



Moving the mouse over the heat map changes the donor and brain structure accordingly. To toggle the sorting by brain structure instead of donor, click on the *Toggle Sort* button in the upper right corner of the heat map.



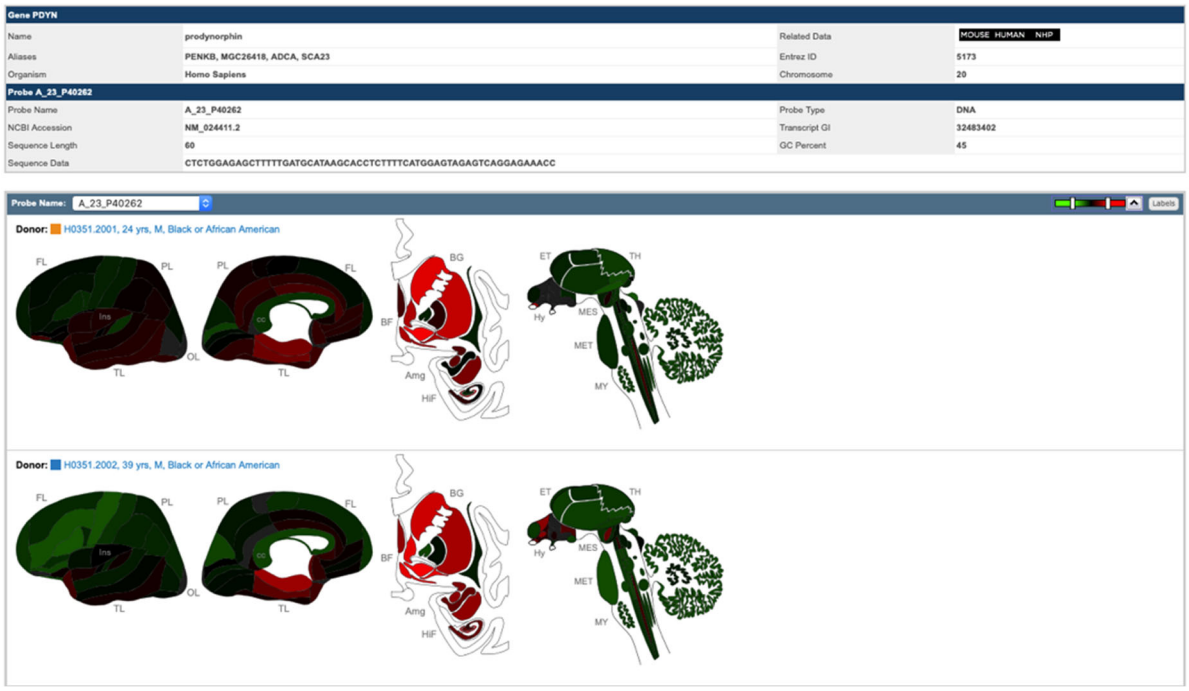
Click the toggle button to enter the structures heat map. Look for patterns of high expression (in the default color view, red - use the toggle at the bottom of the heat map to switch to red/blue colorblind-friendly view if you wish).

The data is presented by z-score, which is the normalized gene expression of each probe over the entire brain of all six subjects. (The z-score is calculated separately for each gene, so you cannot directly compare the z-

scores of two genes and assume they have the same total expression.) To get a coarser look at the brain regions, change the Resolution (at the bottom of the heat map) from Structures to Coarse. Now the heatmap is divided into larger neuroanatomic divisions or regions. Switch back to Structures resolution. *Answer Q3*.

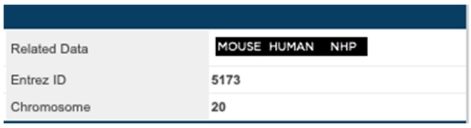
Clicking on a data point in the heat map returns metadata for that probe and brain region. The information is listed above the heat map. Locate a region of high expression (i.e., the nucleus accumbens) and click on that part of the heat map.

Click on the gene symbol (i.e., PDYN, in this example) under Gene Info in the metadata. This brings up additional information about that probe and a series of donor maps. This is the same data as one row in the heat map you were just looking at before but projected onto anatomy.

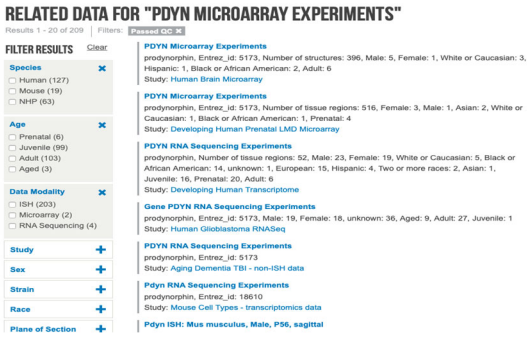


Mouse over the regions of high expression (red) for one of the donors and locate the nucleus accumbens. What is the z-score for this gene in this region? How does it compare to neighboring regions and their z-scores? *Answer Q4*.

If you would like to compare gene expression in humans with that in the mouse, click on the Related Data tab for Mouse in the metadata (upper right).



This brings up a list of related studies in other species. Use the filters on the left to locate studies in the adult mouse and choose Mouse Brain from the Study filter. Next click on a study to see expression patterns in the mouse. *Answer Q5*.

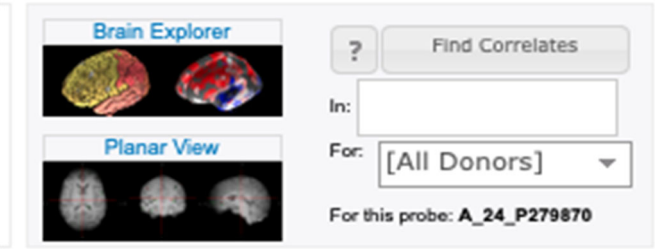


Part II: Other Gene Searches

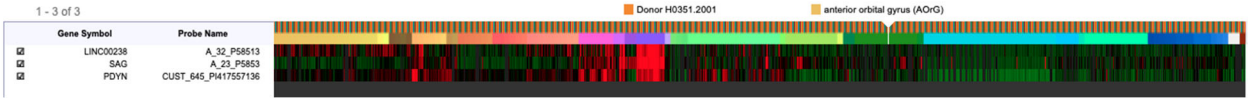
Now let us look for other genes with similar expression patterns to the gene we originally searched for (PDYN). Recall that genes with similar expression patterns may be related in their functions. To do this, return to the microarray heat map for PDYN.

Click on the row for the probe you used before, then select the “Find Correlates” button on the right side of the screen. You can also search for correlates only in selected regions by entering the brain region(s) you are interested in into the search box under the button.

The first few matches will be other probes for the PDYN gene since the correlate search is looking at all probes. Look farther down the list for other genes.

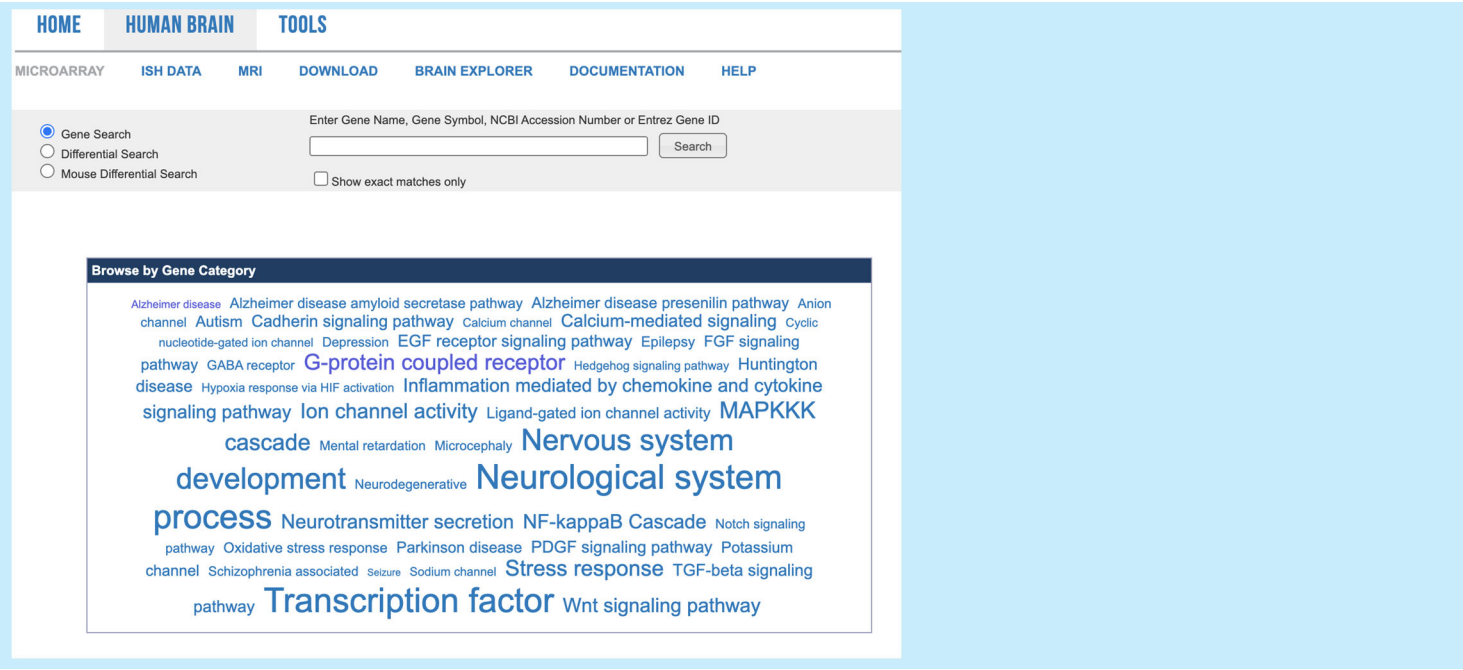


From the new heatmap, select the checkboxes to the left of some new genes you are interested in as well as for PDYN, and click on the View Selection Heatmap button at the bottom. This brings up a new heatmap showing only those genes and their expression patterns.



WE CAN ALSO SEARCH FOR RELATED GENES BY CATEGORY.

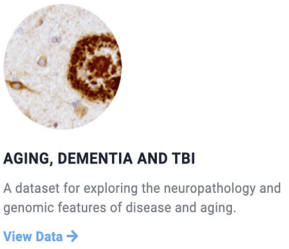
Go back to the Allen Human Brain Atlas homepage (click “Microarray” in the site header). From the list of available Gene Categories, select Seizure. This produces a heatmap for gene expression in genes linked to seizures. *Answer Q6*.



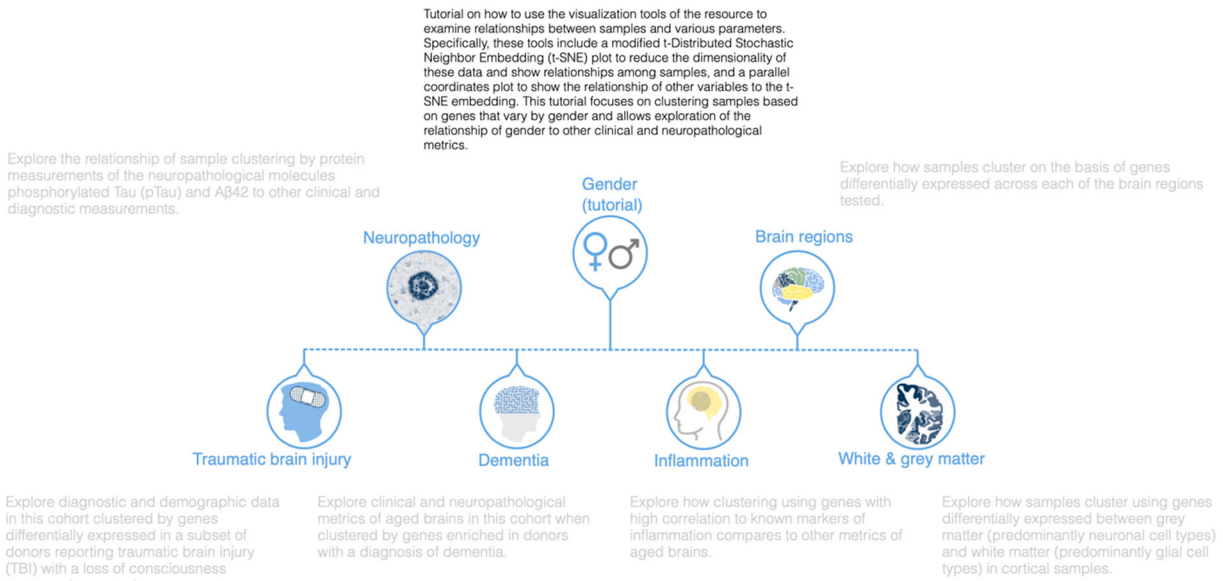
Part 3 Exploring Dementia and Brain Injury

All the brains in the Allen Human Brain Atlas are from healthy young and middle-aged adults. Now we will explore the Aging, Dementia, and TBI database.

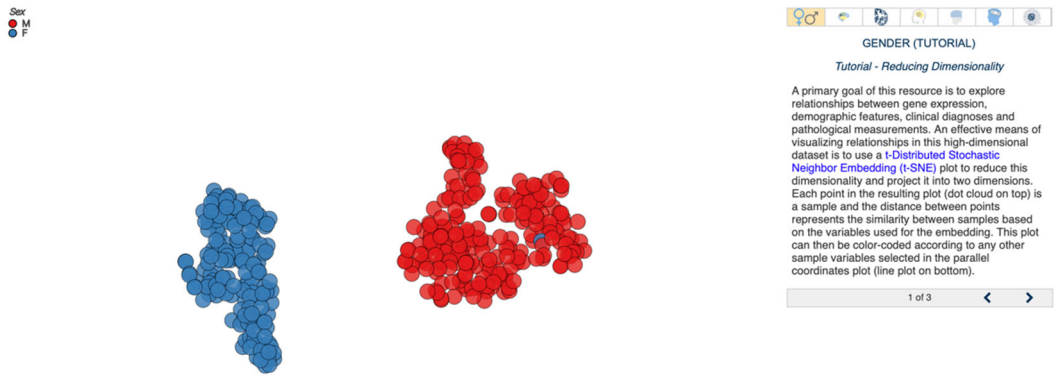
Go to http://portal.brain-map.org and select Aging, Dementia, and TBI from the options available. You can use the drop-down menu at the top of the page or search through the icons for this one:



Select “Overview” in the header to see the page shown below. Begin the tutorial by exploring the gender tutorial to see an explanation of how the data is structured and presented. Click on the Gender icon.



This brings up a new plot like the one shown below: *Answer Q7*.



If you want to learn more about how t-SNE plots work, watch this explainer:

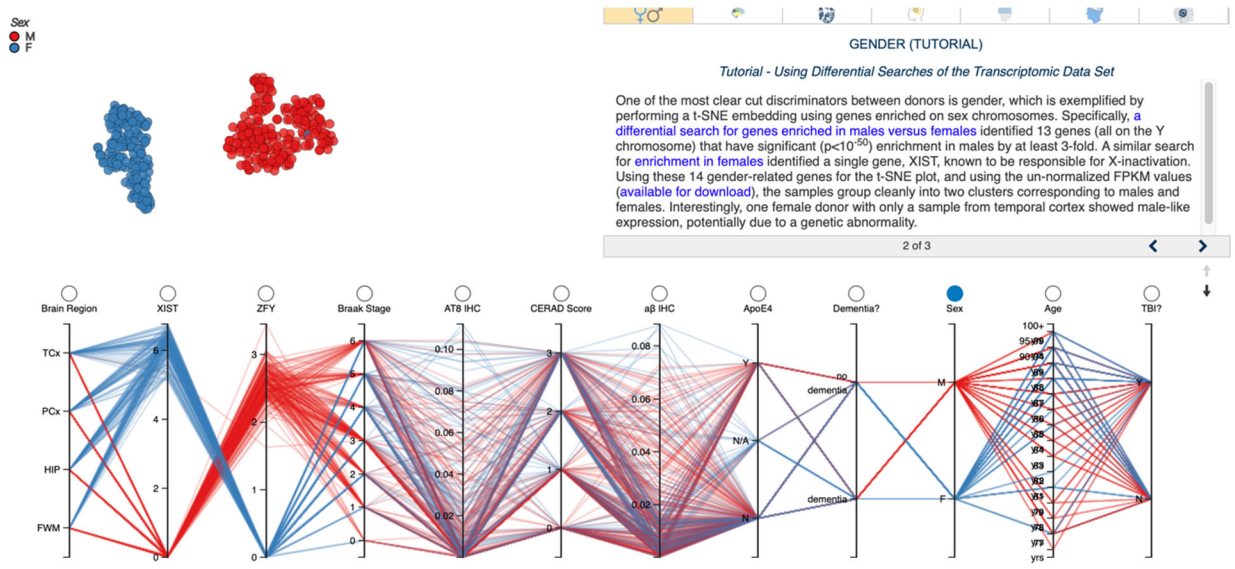
https://www.youtube.com/watch?v=NEaUSP4YerM

Click on the Brain Region button at the bottom to re-color the data by the 4 brain regions (HIP, PCx, TCx, FWM). *Answer Q8*.

Mouse over any of the colored circles in the plot to see a small popup telling the age, evidence of TBI (traumatic brain injury) and dementia status.

Click on the Dementia button along the bottom row. *Answer Q9*.

Now go to page 2 of the gender tutorial. You will see the t-SNE map remains in the upper left and a larger, more complicated set of graphs appears below for the data. This type of plot is called a Parallel Coordinate Plot.



There are 12 data types listed above each axis. The gender data type is selected, so it is used to color code the T-SNE above. Click on the XIST axis to color code the plot by XIST. *Answer Q10*.

Finally, each axis can be modified to show only a subset of the data. For example, click on the Dementia axis and hover your mouse over that axis until it turns gray. Now click and drag to create a slider bar that limits the data represented to just the dementia part of the axis. In the t-SNE plot excluded data points are now colorless circles. *Answer Q11*.

You are now ready to explore the other dimensions of the data more closely. Select one of the dimensions from the menu bar at the top right of the page, above the explanatory text.

When we sort the data by brain region, TBI, dementia state, or other variables, what patterns emerge? *Answer Q12.*

Complete these questions as you work through the tutorial.

1) What is PDYN and what is its function?

2) What is a heat map and how do you read it?

3) What brain regions exhibit high expression of PDYN? You may want to click on the HELP tab and read the section on heatmaps under the Microarray Data.

4) What is the z-score for the nucleus accumbens? What other regions show high expression (z-scores) in all 6 donors?

5) What similarities or differences do you see in expression patterns for PDYN between those in the mouse and those in humans? Hint – use the high-resolution viewer and the anatomical atlas as described in Tutorial 1.

6) What genes are linked to seizure? What are their functions? Do the gene functions make sense? Explain.

7) What does the distance between points represent in the t-SNE plot?

8) Why don't we see the brain regions forming distinct clusters? What brain regions are represented here?

9) Is there any evidence that one sex is more likely to suffer dementia? Explain how you know.

10) What is XIST and why does it cluster distinctly into two separated colors?

11) Which of the 12 data parameters correlate strongly with dementia? How do you know?

12) What patterns do you observe when you sort the data by another dimension? Choose any one and describe what you see.