ADAS-Viewer

Alzheimer's Disease Alternative Splicing

User's Manual
Version 1.0

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Chapter 1 – Introduction

1.1 Overview

ADAS-viewer (Alzheimer's Disease Alternative Splicing) offers a tool for researchers to explore Alzheimer's disease (AD) with multi-omics data in seven brain regions. ADAS-viewer uses RNA-seq and WGS data measured in the cerebellum (CER), temporal cortex (TCX), dorsolateral prefrontal cortex (DLPFC), frontal pole (FP), inferior frontal gyrus (IFG), parahippocampal gyrus (PHG), and superior temporal gyrus (STG) which was generated from three independent cohorts of the Accelerating Medicines Partnership-Alzheimer's Disease (AMP-AD) project: the Mayo Clinic, ROS and MAP (ROSMAP), and Mount Sinai Brain Bank (MSBB). In addition, ADAS-viewer utilizes methylation and miRNA data measured in the DLPFC region by ROSMAP. ADAS-viewer also includes data from cognitively normal old adults and AD patients; in CER and TCX by Mayo Clinic which was measured in cases of progressive supranuclear palsy (PSP) and other pathologic aging diseases. Inclusion of clinical information such as sex, ethnicity, diagnosis, plaque mean, and Braak stages allows ADAS-viewer to provide a wide range of analysis of phenotypic characterizations (AD vs. normal, sex-specific molecular traits in AD, AD vs. normal with dementia, or Braak stage associated transcripts/AS exons in AD) that are of current interest to researchers.

1.2 Environment

ADAS-viewer is compatible with most types of web browsers in Windows, Linux, and Mac OS. As long as your OS has the latest versions of any web browser, you can freely use *ADAS*-viewer. There is a list of web browsers and the versions that we tested and are compatible. Please contact with us at issue.leelab@gmail.com if you have any technical issues or questions on using *ADAS*-viewer.

ADAS-viewer is compatible with any of the web browsers and their versions for Windows, Linux, and Mac as follows:

- Chrome version 56.02924.87 (64-bit)
- Firefox version 52.0.2 (64-bit)
- Internet Explorer 11.953.14393.0 (64-bit)
- Microsoft Edge 38.14393.0.0 (64-bit)
- Safari 10.1 (64-bit)

Chapter 2 – Get Started

2.1 Homepage

ADAS-viewer is available at http://genomics.chpc.utah.edu/AD/ (**Figure 1**). The homepage consists of the ADAS-Viewer logo symbolizing the ADAS-viewer at the top, links to easily navigate between web pages (about, search, data, tutorial contact). You can return to the *ADAS*-viewer homepage by clicking on the ADAS-Viewer logo at the top of the webpage or by clicking the "About" link.

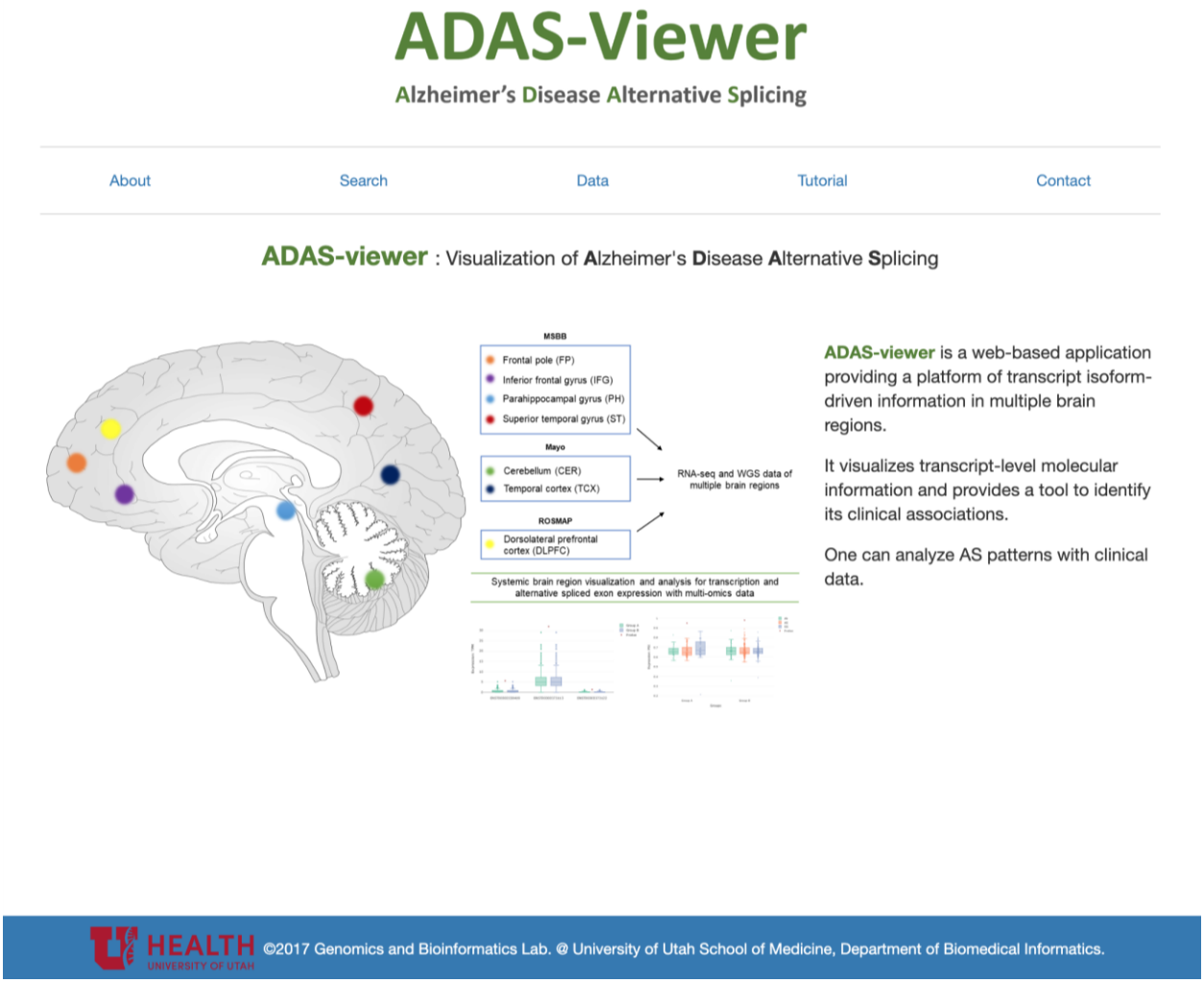


Figure 1. The homepage of *ADAS*-viewer

2.2 Keyword Search

User should go to the search page by clicking on the Search link to explore AD with multi-omics data in seven brain regions.

Search STEP 1.

: Enter the keyword in the form field and click the button (Figure 2)

As an input keyword for *ADAS*-viewer,

1. the HUGO-approved gene name (e.g., TREM2)
2. Ensembl gene ID (e.g., ENSG00000120885)
3. SNP (rs number, e.g., rs7982)
4. DNA methylation number (cgID, e.g., cg00000236,)
5. miRNA ID (e.g., hsa-mir-139-5p)

are searchable for an alternatively spliced gene. The next page displays the search results for alternatively spliced genes that match the input keyword (**Figure 2**). For miRNAs, multiple genes can be listed, because one miRNA targets multiple genes.

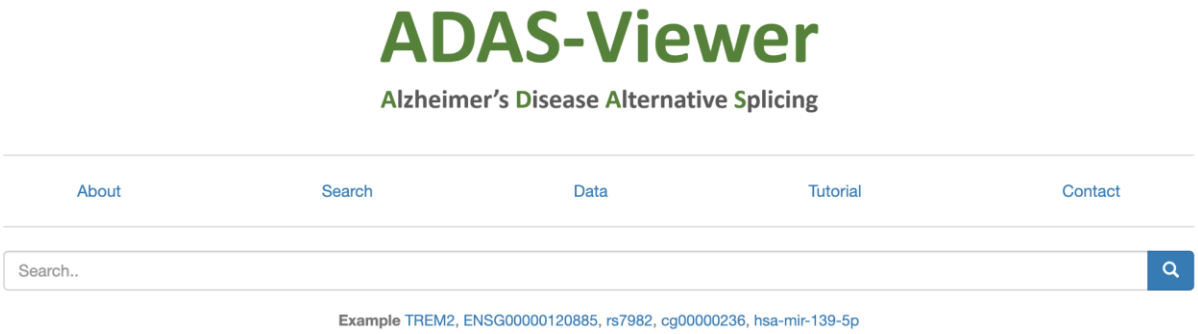


Figure 2. Search tab for alternative splicing (AS) genes

Search STEP 2. : Check the "Alternatively Spliced Gene found for Gene:" XXXX "" message and click on the link of the gene of interest (Figure 3)

As an example, the gene symbol “abca7” is input, resulting in the “Alternatively Spliced Gene found for Gene: “ABCA7” message and the a link to “ABCA7”(**Figure 3**). User can click on the “ABCA7” link to proceed to the next step. If no gene is found, the message "No Result" will be displayed instead.

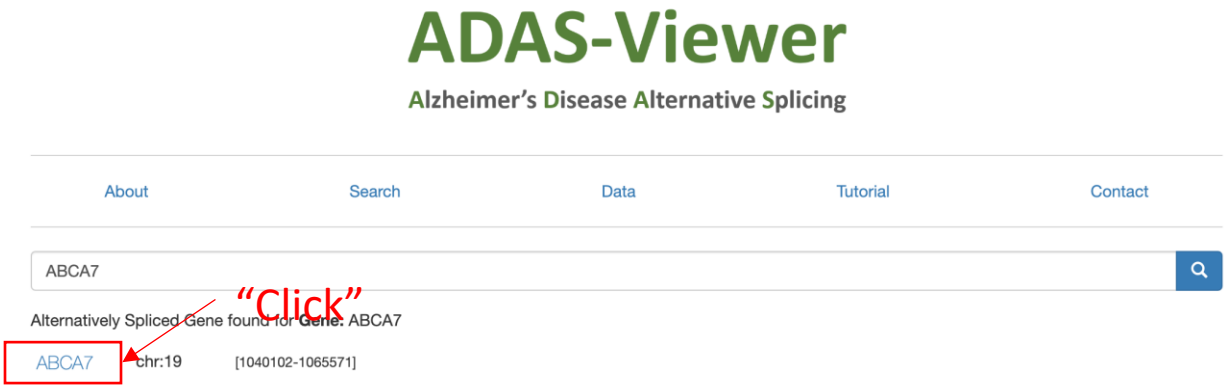


Figure 3. AS gene search result (e.g., *ABCA7*)

Chapter 3 – AS Gene(transcript) Navigator

After searching the gene via keyword search and clicking the gene link, a web page will be created showing the following:

1. AS Gene Navigator (**Figure 4**)
2. Transcript expression in brain regions tab (**Figure 5**)

! All tabs used in *ADAS*-viewer have a three-sided border and the black ones are selected. For example, here Transcript expression in brain regions Analysis the "Transcript expression in brain regions" tab is selected by clicking.

3.1 AS Gene(transcript) Navigator

Figure 4 is a screenshot of AS Gene Viewer for the *ABCA7* gene, which is seen after clicking "*ABCA7*" as shown in **Figure 3**. This gene has 17 transcript isoforms, comprised of 84 distinct exons in total. The blocks and lines represent the exons and introns (**Figure 4**). Among exon blocks, thicker blocks are the coding regions (CDS), and thinner blocks are untranslated regions (UTRs) (**Figure 4**).

3.2 Exon Usage Track

The transcript track named "Exon usage" in **Figure 4.** on the left side is composed of the representative exons that are defined by clustering overlapping exons. Exons are clustered according to the genomic location to find overlapping exons. Users are able to visualize representative exons, which is essential a concatenation of the longest exons in each exon cluster. Exons whose length differs from the representative exon can be recognized easily to be alternatively spliced (i.e., 5' and 3' splice sites and intron retention). The color on the exon represents the skipping frequency of each exon; the lighter the color, the more frequently it is skipped. The user can also see the same information through the mouse over the pop-up for each exon that shows how many transcripts missed the given exon.

3.3 SNP, Methylation and miRNA Track

SNPs, methylations, and miRNAs that exist within the defined transcribed region of a gene are also visualized in this view (**Figure 4**).

3.4 Intron Scaling and Useful Features

An intron scale of 0% is a transcript viewer in the mRNA coordinate that indicates splicing features (**Figure 4**). An intron scale of 100% makes the viewer equivalent to a genome browser, which is convenient for specifying the genomic features in introns.

At the top right of all AS Gene (transcript).

Navigators have the following symbols:

zoom in, zoom out, reset axes, save .jpg, and save .svg from left to right.

Figure 4. AS gene(transcript) navigator (e.g., *ABCA7).*

3.5 Select an exon

When you click an exon of interest, the selected exon is highlighted in green (**See Figure 7**). The exon selection in AS Gene Navigator pre-divides the transcripts into two groups: transcript(s) with exon skipping and transcript(s) without exon skipping as a default. The two groups are by default in the 'Group a Transcript' Option, but the user can re-group the transcripts under Option (**See Section 4.1 Group a Transcript).**

3.6 Transcript expression in brain regions tab

There are two tabs below the AS Gene Navigator section, one is a the "Transcript expression in brain regions" tab set by default and the other is the "Analysis" tab. In the "Transcript expression in brain regions" tab, the expressions of normal and AD group for a given transcript were visualized through a heatmap (left) in the brain image and through a boxplot (right) in the seven brain regions. In the heatmap, different shades were used to show the average TPM values of each brain region. A color key for the heat map is shown on the right. You can view the expression of the transcripts by clicking on the spliced transcripts (e.g., ENST...) ID box of the gene (**Figure 5**). Also, you can get additional information from the popup window by hovering over the heatmap section of the brain regions and the sample points on the boxplot.

The Transcript expression in brain regions tab contains the following useful features:

From the left, there are plot save, zoom function, move function, zoom in, zoom out function, auto scale function and reset axes function.

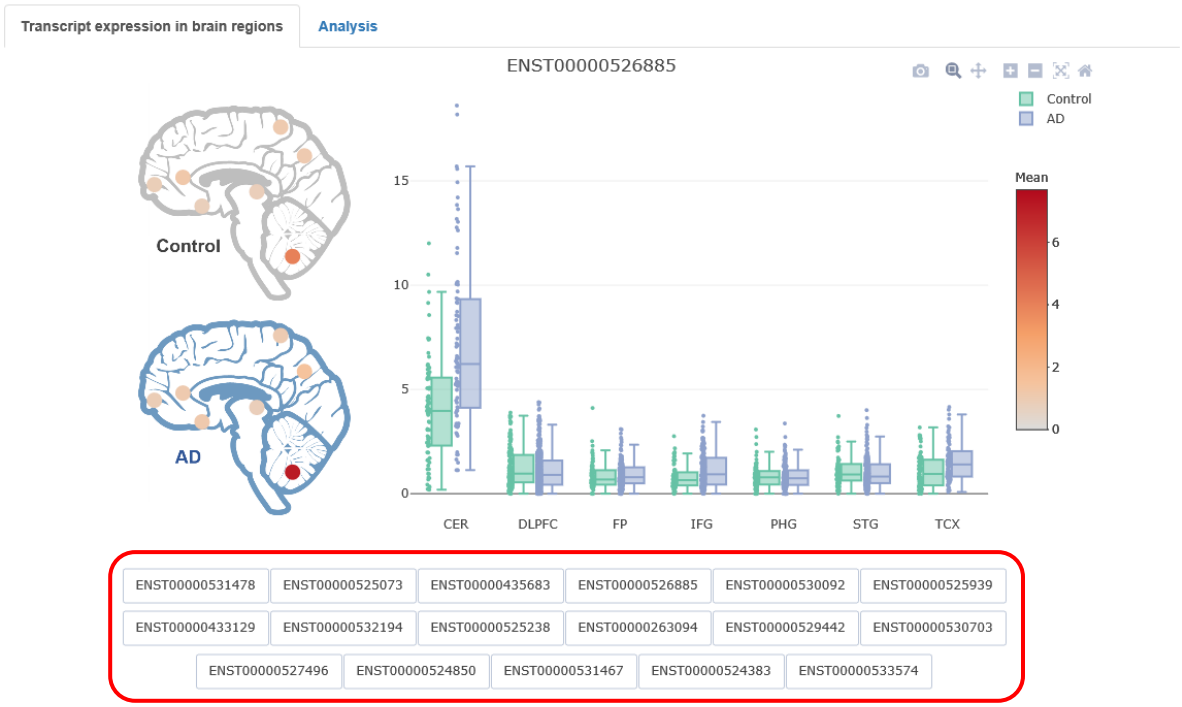


Figure 5. Transcript expression in brain regions tab The expressions of normal and AD group for a given transcript were visualized through a heatmap (left) in the brain image and through a boxplot (right) in the seven brain regions.

! If the Transcript boxes (i.e., red box in the **Figure 5**) are displayed at the bottom of the plot in ADAS-viewer (**Figure 5)**, by default the plot for the leftmost, upper Transcript is created. If you want to plot another transcript, click the box and check the ENST ID of the transcript at the top of the plot.

Chapter 4 – Option

Given the AS gene selected by the user in the “Search” stage (**Figure 3**) and a specific brain region, the user can set up a case 1 and case 2 group for comparative analysis. To do this, users can click on the “Analysis” tab.

4.1 Analysis tab

The Analysis tab is located next to the Transcript expression in brain regions tab. Analysis tab is composed of 1. Option part and 2. Result part. If you define a group that you want to compare in the "Option" part, the outcome is shown in the "Result" part.

1. Option part
   A. Step2 Select transcripts
   B. Step3 Select a case

2. Result part
   A. Comparisons
   B. SNP
   C. Methylation
   D. miRNA

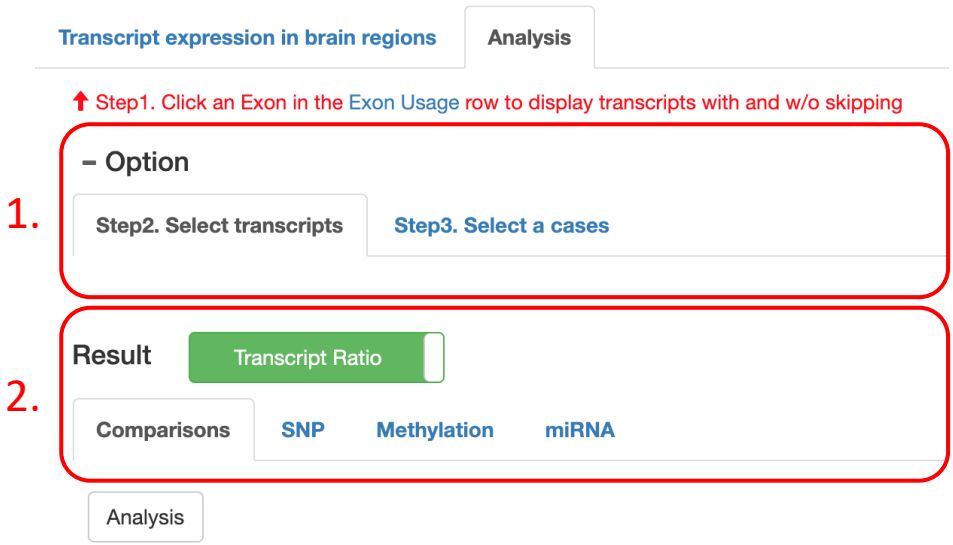


Figure 6. Analysis tab

4.2 Group a Transcript

Option STEP 1.

: Click an Exon in the Exon Usage row in the AS Gene Navigator

Double click the exon located in the "exon usage" line in the AS Gene Navigator at the top of the web page. The selected exon is marked in green **(Figure 7)**. When an exon is selected, the exon is divided into two groups: skipping transcript and non-skipping **transcript (Figure 8)**.

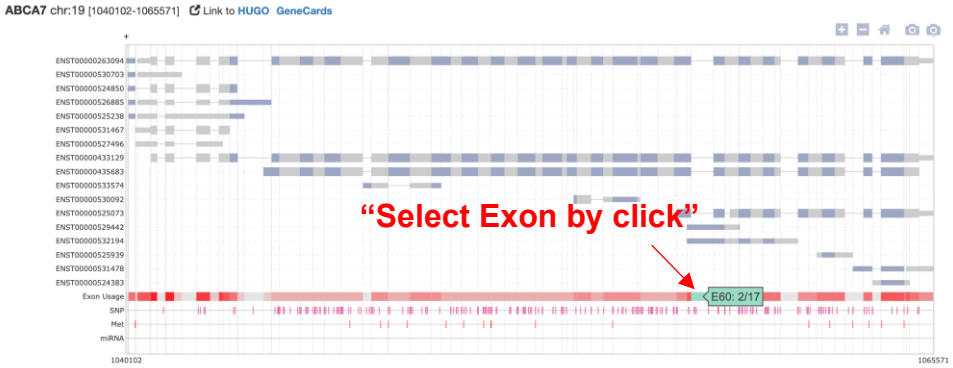


Figure 7. Selecting an exon by clicking it in the AS gene navigator.

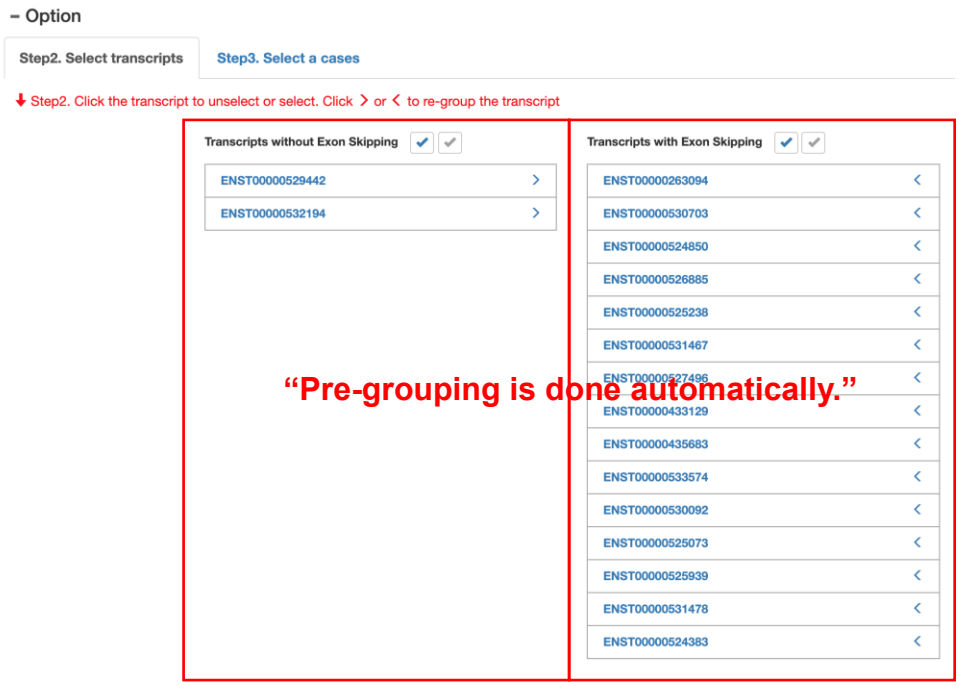


Figure 8. Pre-grouping the transcripts according to whether the selected Exon is skipped.

Option STEP 2.

: If you want to adjust the group, you can move the transcript with the “>” or “<“ button or you can exclude the transcript by clicking.

The user can further re-group the transcripts by 1) clicking the transcript id to unselect the pre-selected transcript or 2) clicking “>” or “< “to move the transcript into another group. **Figure 9** shows the regrouping options and regrouped transcripts, respectively. The ratio of differential expression between the two transcript groups will be calculated and plotted in the result panel.

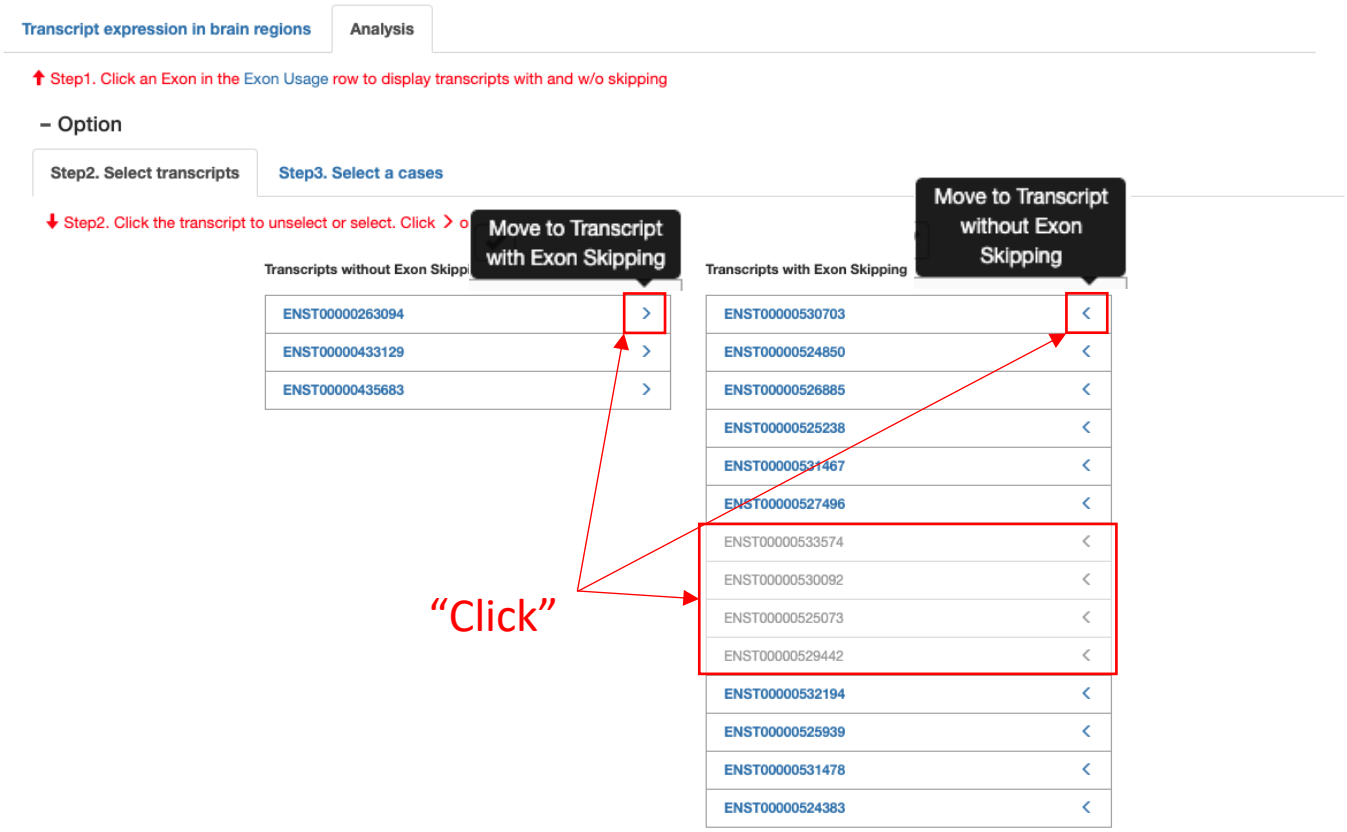


Figure 9. Option: group a transcript. Using the “>” or “<” buttons or deselecting, transcripts can be regrouped.

4.3 Group a Case

Option STEP 3.

: Click on one of the seven brain regions and check or uncheck the conditions for each group.

The user can select a case according to the clinical information for brain regions (**Figure 10**). Seven brain regions are available in the option. Users define clinical features and brain regions in two groups to find meaningful percent spliced in (PSI) or expression patterns. This option helps users obtain evidence on which transcript isoforms (or exons) are important to the clinical outcome.

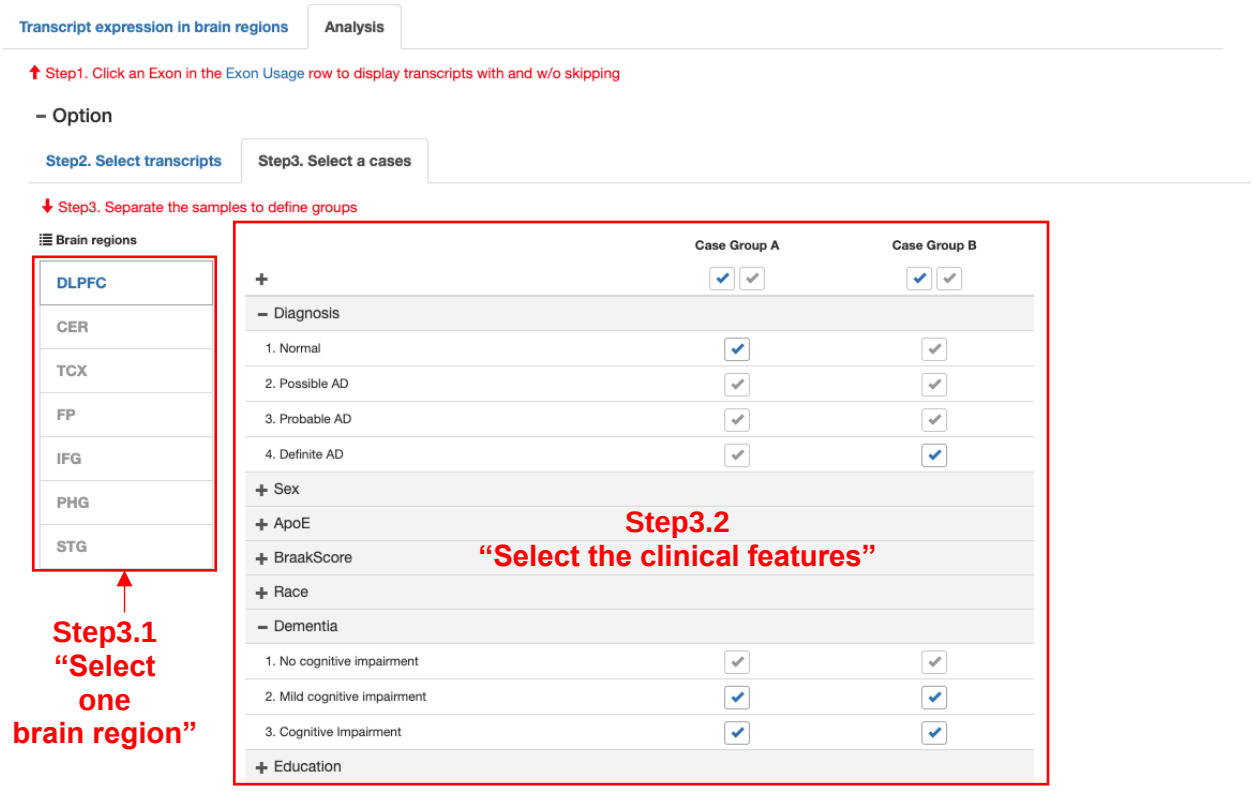
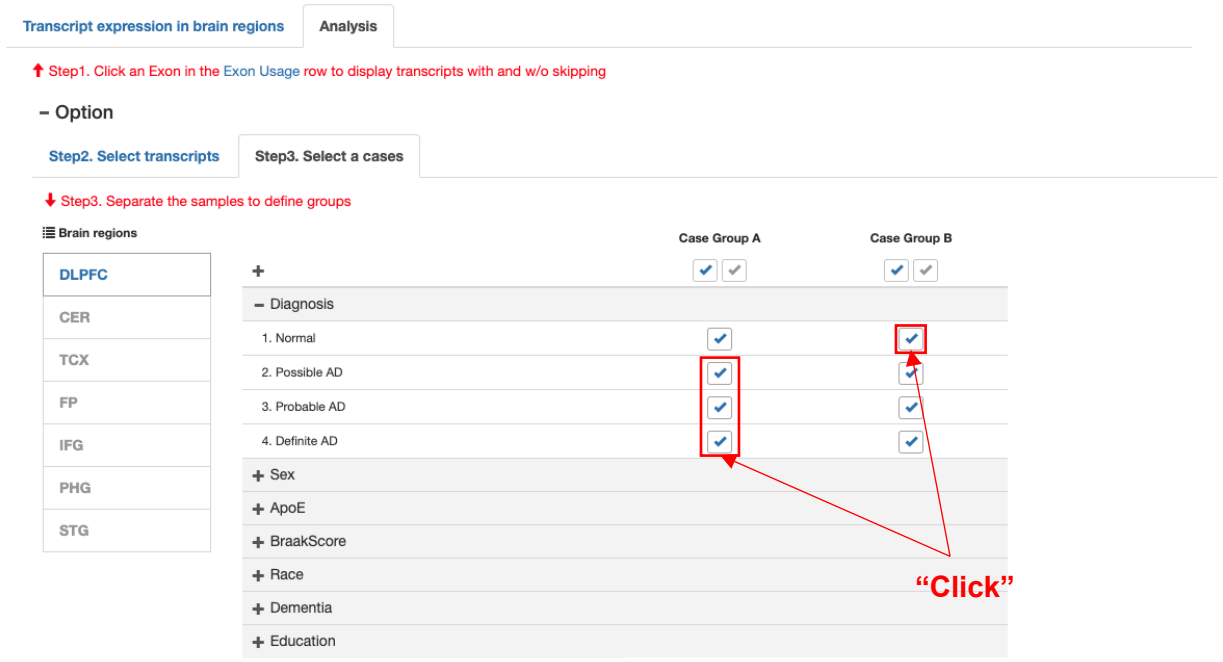


Figure 10. Option: group a case. The left panel lists 7 brain regions. Once a user selects a brain region, the right panel shows the clinical features of the selected region, allowing the user to select two cases to be tested in the output.

! As shown in **Figure 11**, when setting clinical features, the ☑ blue check box is checked in the corresponding item, and the ☑ gray check box is excluded. And you can select all or deselect all by using the ☑ ☑ checkboxes below each of "Case Group A" and "Case Group B" at the top.

(a)



(b)

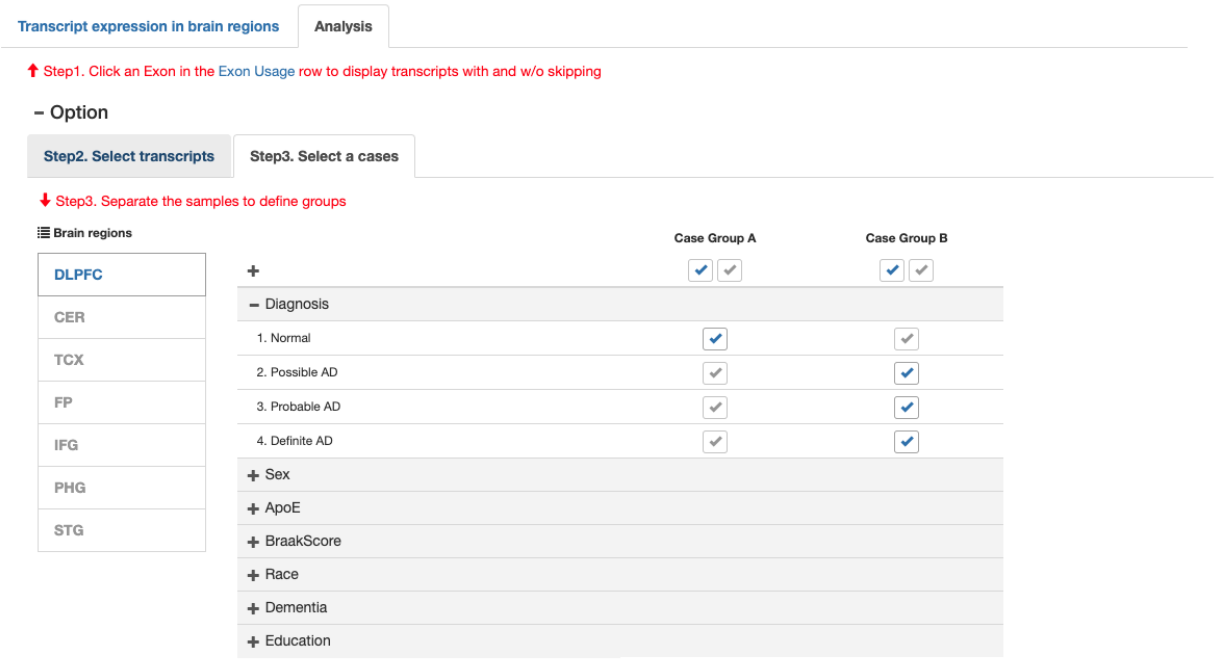


Figure 11. Example of two groups of cases selected in a demonstration of output analysis. (**a**) In the left panel, the use clicks one brain region and then clicks clinical features in 'case inclusion' option. (b) The output will now analyze normal vs. possible/probable/definite AD.

Chapter 5 – Result

Result STEP 1

: If you want to compare PSI values between groups, you can select Transcript Ratio

On the other hand,

If you want to compare expression level between groups, you can select Transcript Expression

! Transcript Ratio and Transcript Expression can be switched by clicking.

Result part is comprised of four components for plotting the results:

1. Transcript Comparison
2. SNPs
3. Methylation
4. miRNA

5.1 Transcript Comparison

In comparisons, users can identify differential expression between two groups users separated with clinical information.

Result STEP 2.1

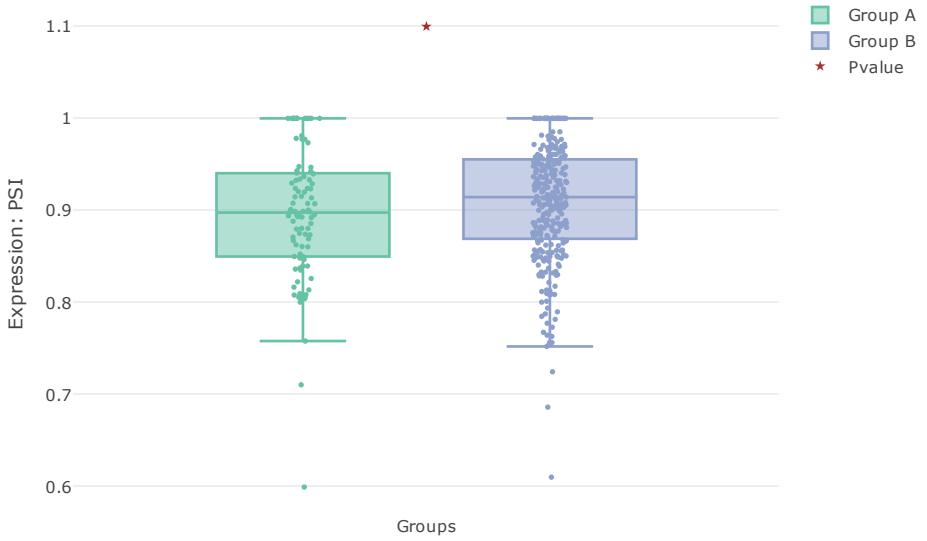
: 1. Re-check Transcript Ratio ***an*** Transcript Expression ***or by clicking on it***

2. Click Analysis ***the button.***

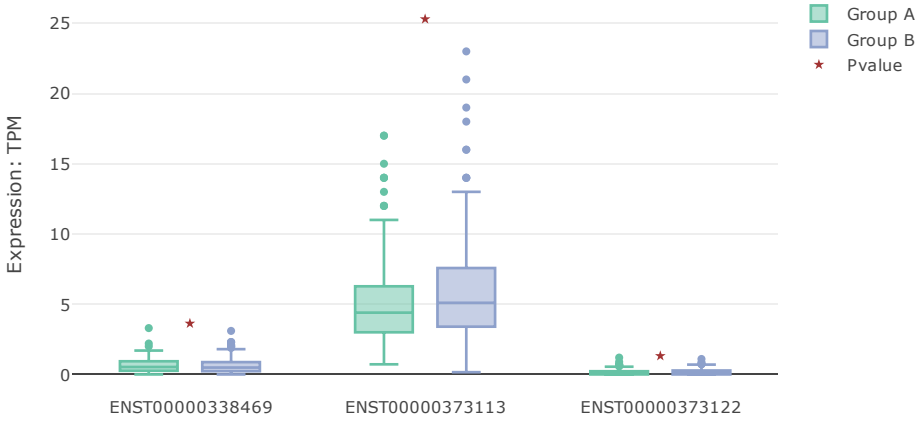
! The effect of the Transcript Ratio and Transcript Expression transitions is reflected only by clicking the Analysis button.

The results are illustrated in the boxplot, showing the distribution of PSI or TPM values between two groups of cases, which is user-defined in the “Group a Case” Option. The PSI value refers to the differential expression between two groups of transcripts. If you select the “Transcript Ratio” button (Figure 9a) next to the subtitle “Result” and click “Analysis”, the result of PSI value is displayed. If you change to “Transcript Expression” and click “Analysis” **(Figure 6)**, the result is the TPM value. In **Figure 12a and 12b**, the Y-axis is PSI value, ranging from 0 to 1, and the X-axis is the case. The plot shows dots that you can mouse over for detailed clinical information on each case **(Figure 13)**. Significant p-values between the two groups are marked with an asterisk **(Figure 14)**. Under the plot, the table summarizes the number of cases for each group and the common cases in both groups, followed by the selected clinical features for each group **(Figure 12c)**.

(a)



(b)



(c)

Patient Group A: 95, **Patient Group B:** 307, **Common:** 0

Case Group A	Diagnosis: 1. Normal, 2. Possible AD, 3. Probable AD, 4. Definite AD Sex: **Female**, Male ApoE: **E2E2, E2E3, E2E4, E3E3, E3E4, E4E4, Unknown** BraakScore: **0, 1, 2, 3, 4, 5, 6** Race: **Asian or Pacific Island, Black, Negro, African-American, Native American, Indian, White** Dementia: **1. No cognitive impairment, 2. Mild cognitive impairment, 3. Cognitive Impairment** Education: **1. Elementary, 2. High School, 3. College, 4. Graduate and professional**
Case Group B	Diagnosis: 1. Normal, 2. Possible AD, 3. Probable AD, 4. Definite AD Sex: **Female**, Male ApoE: **E2E2, E2E3, E2E4, E3E3, E3E4, E4E4, Unknown** BraakScore: **0, 1, 2, 3, 4, 5, 6** Race: **Asian or Pacific Island, Black, Negro, African-American, Native American, Indian, White** Dementia: **1. No cognitive impairment, 2. Mild cognitive impairment, 3. Cognitive Impairment** Education: **1. Elementary, 2. High School, 3. College, 4. Graduate and professional**

*Options selected by both groups appear in **boldface**.

Figure 12. Transcript comparison (**a**) Transcript Ratio (PSI) result. (**b**) Transcript Expression (TPM) result. (**c**) User-defined group property table.

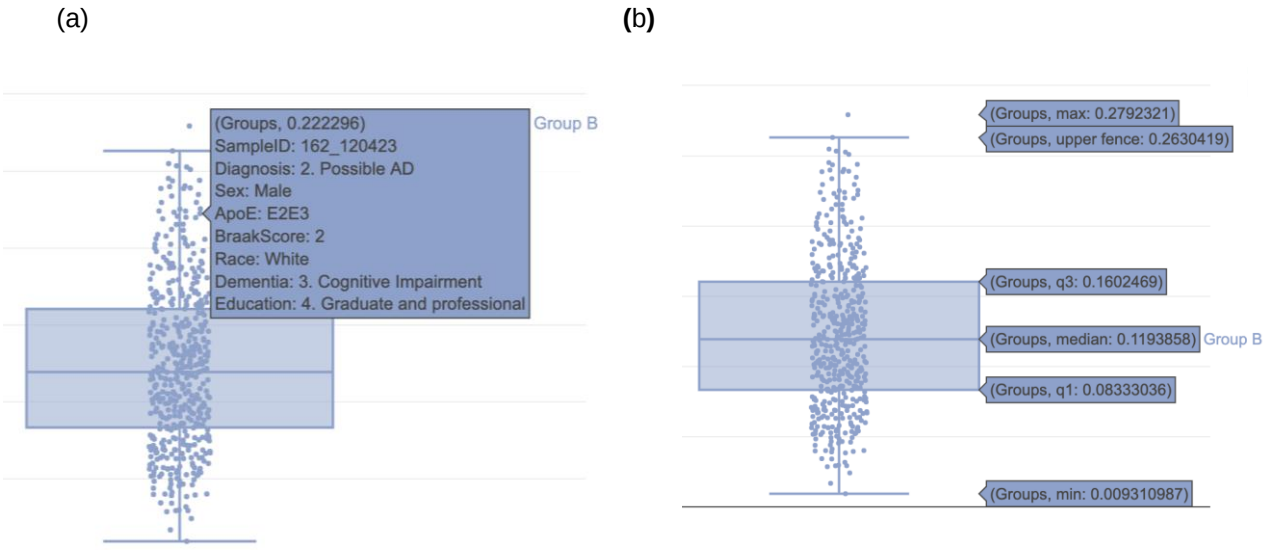


Figure 13. Boxplot in *ADAS*-viewer (a) Hover your cursor over each point on the boxplot to display a popup window with sample information. (b) If you move the cursor to the boxplot, a popup window containing section information will appear.

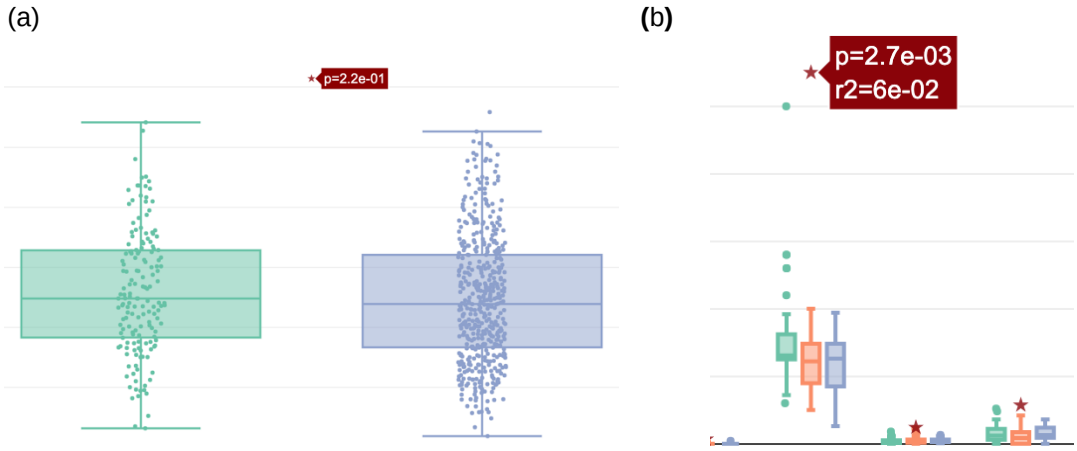


Figure 14. Asterisk in boxplot (a) p-value (b) p-value and r-square values.

5.2 SNPs

The SNP analysis is to identify eQTL and sQTL by combining transcript/exon expression with SNP genotype.

Result STEP 2.2

: 1. Select an Transcript Ratio ***or*** Transcript Expression ***by clicking on it***

2. Select the SNP ***tab***

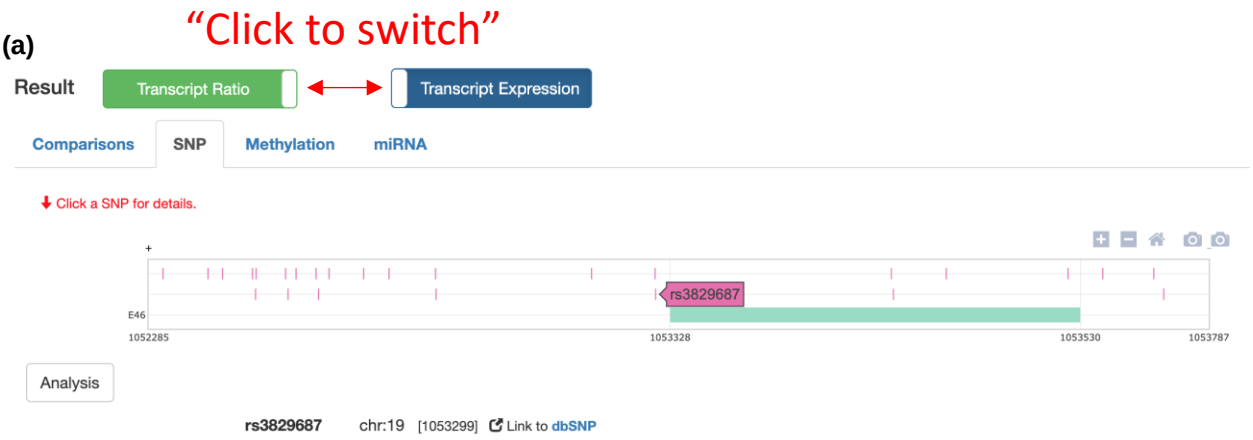
3. click SNP (Pink series vertical bar) on the AS Gene Navigator (Figure 15a).

4. confirm that the SNP information appears at the bottom

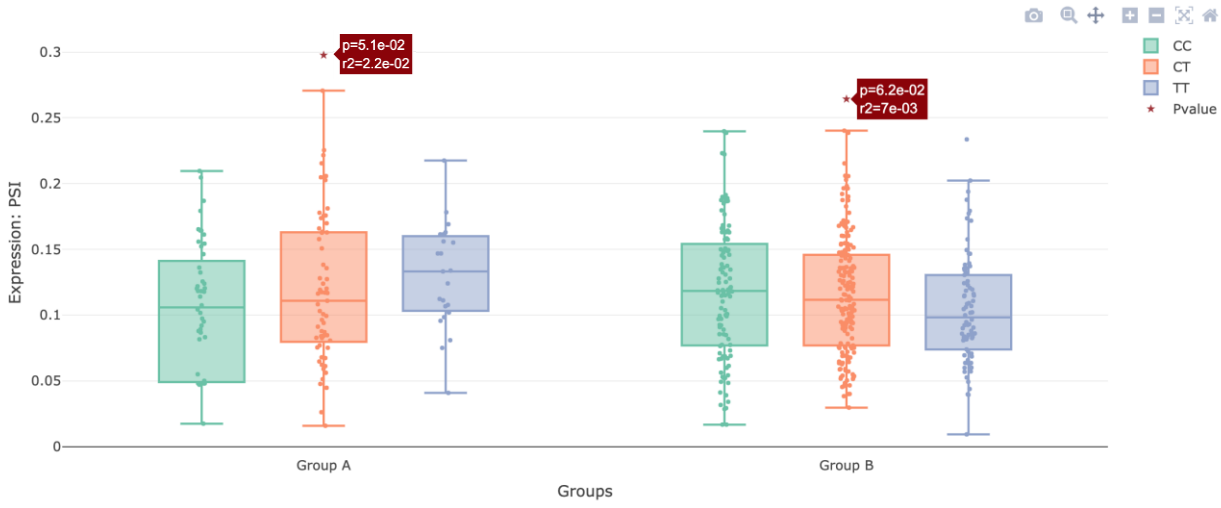
5. press the Analysis ***button.***

Once a user clicks a certain exon in AS Gene Navigator, this panel automatically displays the zoomed-in view of the selected exon, including the right (downstream) and left (upstream) adjacent introns and all SNPs that exists within the displayed genomic region. When a user clicks a point of SNP(RSID) and the “Analysis” button, it will show boxplot results by genotype **(Figure 15)**.

(a)



(b)



(c)

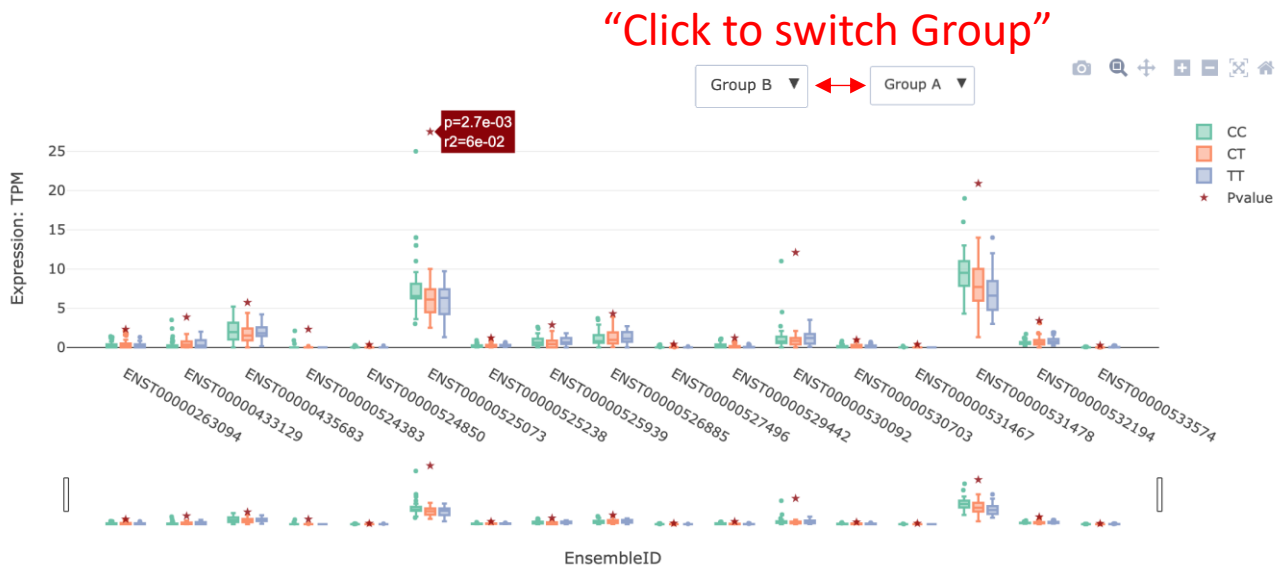


Figure 15. SNPs results (a) AS Gene Navigator SNP (**b**) Transcript Ratio (PSI) (**c**) Transcript Expression (TPM).

5.3 Methylation

The methylation analysis is to perform the linear regression to correlate transcript/exon expression with methylation status.

Result STEP 2.3

: 1. Select the Methylation ***tab***

2. click Methylation (Red series vertical bar) on the AS Gene Navigator (Figure16a).

3. confirm that the Methylation information appears at the bottom

4. press the Analysis ***button.***

Once a user clicks a certain exon in AS Gene Navigator, this panel automatically displays the zoomed-in view of the selected exon, including the right (downstream) and left (upstream) adjacent introns and all methylation that exists within the displayed genomic region. When a user clicks a point of methylation and the "Analysis" button, the plot summarizes the analysis by showing the distribution of cases according to PSI value **(Figure16b)** or TPM value **(Figure16c)** on the y-axis and methylation status on the x-axis. The mouse over pop-up on the dot displays the selected clinical features for a case. If a case is common in two groups of cases, the dots overlap. The legend of the plot is clickable, appearing and disappearing with the corresponding distribution of cases in the plot area (See **Figure 17c**).

Figure 16. Methylation results (a) AS Gene Navigator methylation (b) Transcript Ratio (PSI) (c) Transcript Expression (TPM).

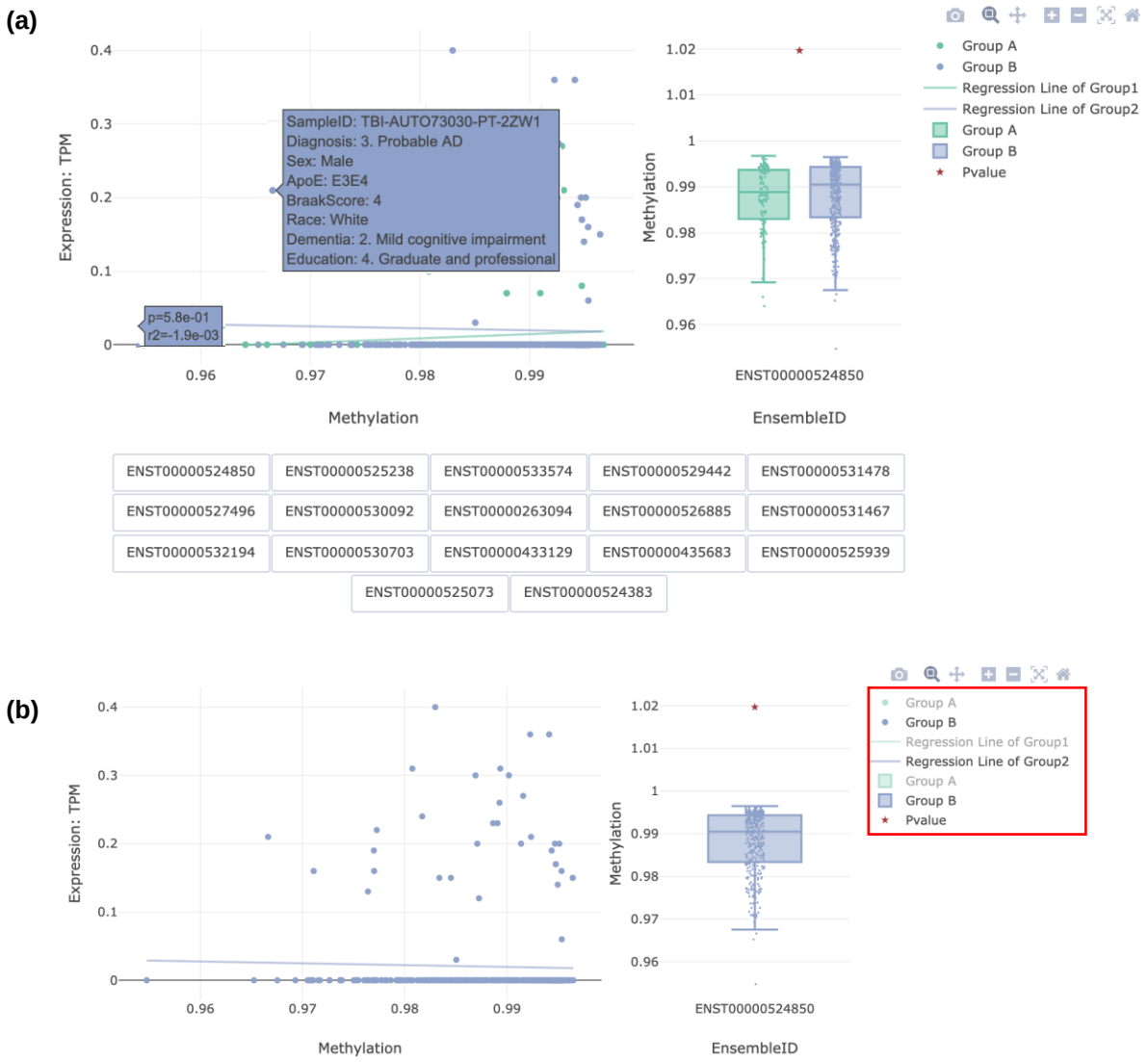


Figure 17. Dynamic features of regression plot (a) If you move the cursor to the regression curve, a popup window containing p-value and R-scale values will appear. (b) You can also click on the legend in the red box to see only plots of a specific group. For example, in (b), only Group B was selected.

5.4 miRNA

The miRNA analysis is to perform the linear regression to correlate transcript/exon expression with miRNA expression.

Result STEP 2.4

: 1. Select the miRNA ***tab***

2. click miRNA (Blue box or gray box) on the AS Gene Navigator (Figure18a).

- ***The blue box is for the miRNA user selected as the input value in the 2.2 Keyword Search step, and the gray box is the miRNA near the selected exon.***

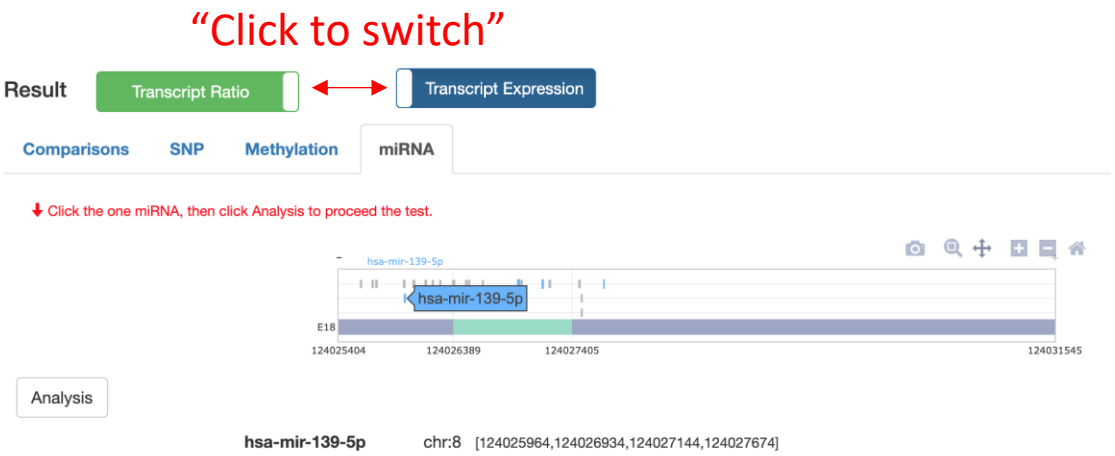
3. confirm that the miRNA information appears at the bottom

4. press the Analysis ***button.***

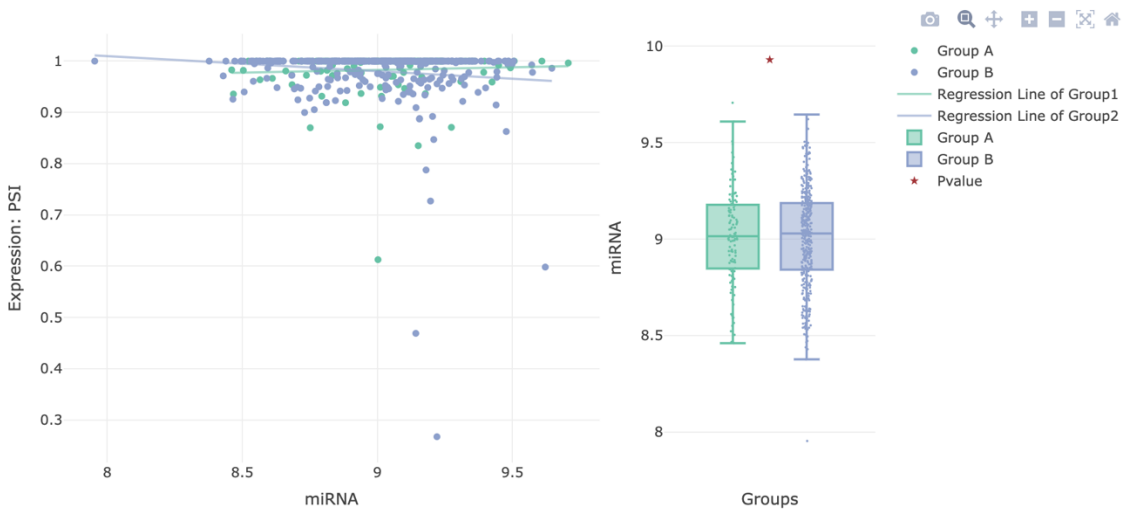
Once a user clicks a certain exon in AS Gene Navigator, this panel automatically displays the zoomed-in view of the selected exon and all miRNA binding sites that exist within the displayed genomic region. When a user clicks a miRNA and the “Analysis” button, the plot summarizes the regression analysis by showing the distribution of cases according to PSI values on the y-axis and miRNA expression on the x-axis (**Figure 18b**). The mouse over pop-up on the dot displays the selected clinical features for a case. If a case is common in two groups of cases, the dots overlap. The legend of the plot is clickable, appearing and disappearing with the corresponding distribution of cases in the plot area (See **Figure 17c**)

.

(a)



(b)



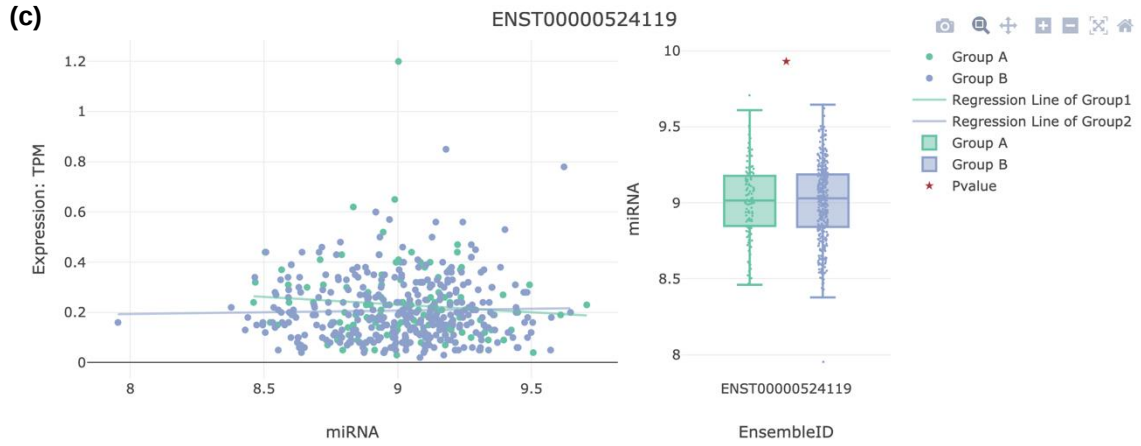


Figure 18. miRNA results (a) AS Gene Navigator miRNA (b) Transcript Ratio (PSI) (c) Transcript Expression (TPM).