



Center for Diversity in
Public Health Leadership Training
at Kennedy Krieger Institute

A Pilot Study: Exploring The Association Between Wildfire Exposure And Placental DNA Methylation

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INTRODUCTION

There is some evidence that exposure to air pollution causes toxicity in the body in addition to having an association with the risk of autism spectrum disorder (ASD) if there is prenatal exposure to pollutants¹. Wildfire rates in California have been increasing in the past several years, and there have been many studies suggesting respiratory and immune impacts. However, there have not been many studies on the molecular impact of wildfires (WF) on the placenta, that could serve as potential biomarkers of the potential harm to the fetus and the expectant mother. In this study, we examined the association between WF exposure and DNA methylation using R programming language, an R package called Comethyl and predict the effects of DNA methylation over gene bodies in relation to neurodevelopment and immune disorders.

OBJECTIVE

In this study, our objective is to identify any modules of gene body comethylation in placenta DNA that significantly associates with maternal wildfire exposure. If such modules are identified, we will investigate their membership to identify potential candidate genes for the developmental effects of prenatal wildfire exposure.

METHODS

- Cyberduck was used to access gene clusters on servers².
- Comethyl, an R package, was used to identify and interpret modules of comethylated regions from whole-genome bisulfite sequencing data. The modules were identified and explored by using sample traits and examining functional enrichments. Results were visualized using ggplot2, another R package, used for creating graphics using R studio program³.
- R studio was used for an easier coding interface better visualization⁴.
- From a total of 126 samples used to generate comethylation modules, 11 of them were exposed to WF. Figure 1.0 shows the wildfire trait selected for and the two modules selected- module magenta and module salmon, and correlation maps and scatterplots were made using ggplot2.

RESULTS

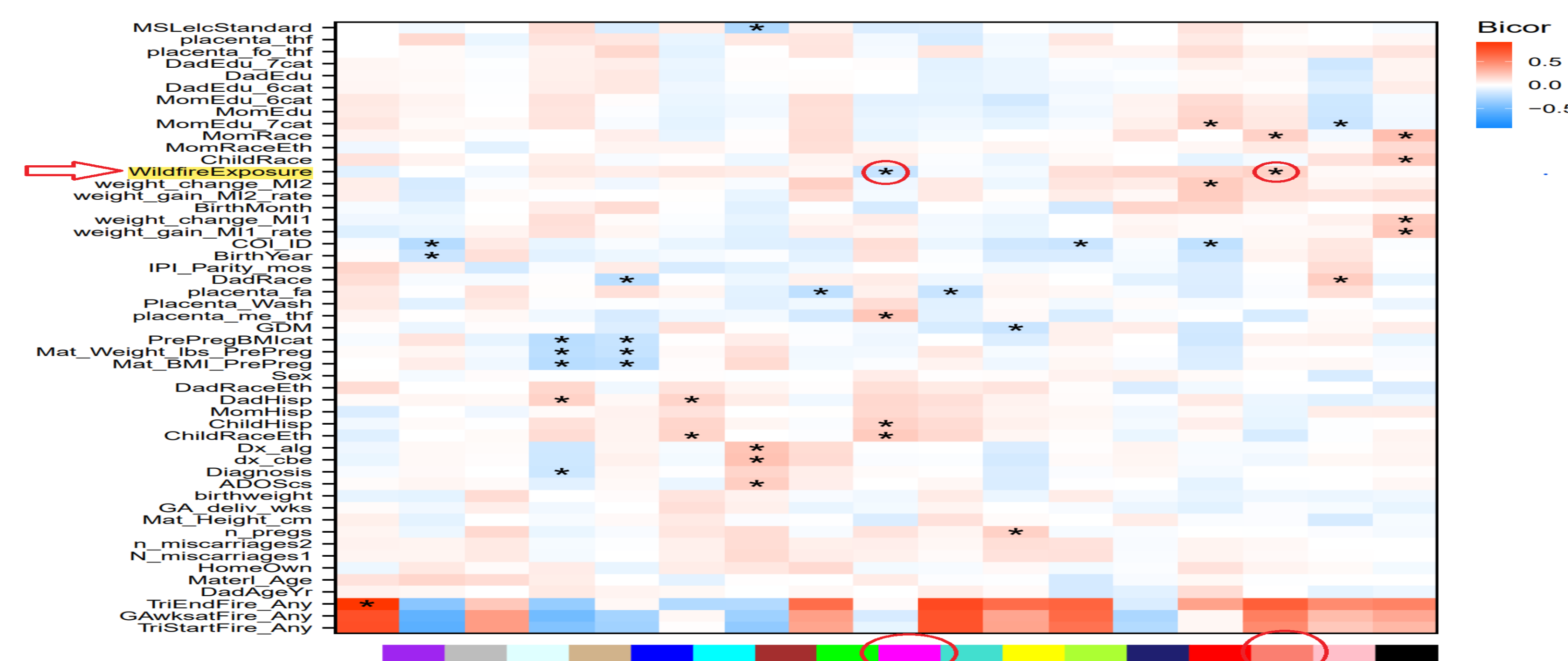


Figure 1.0

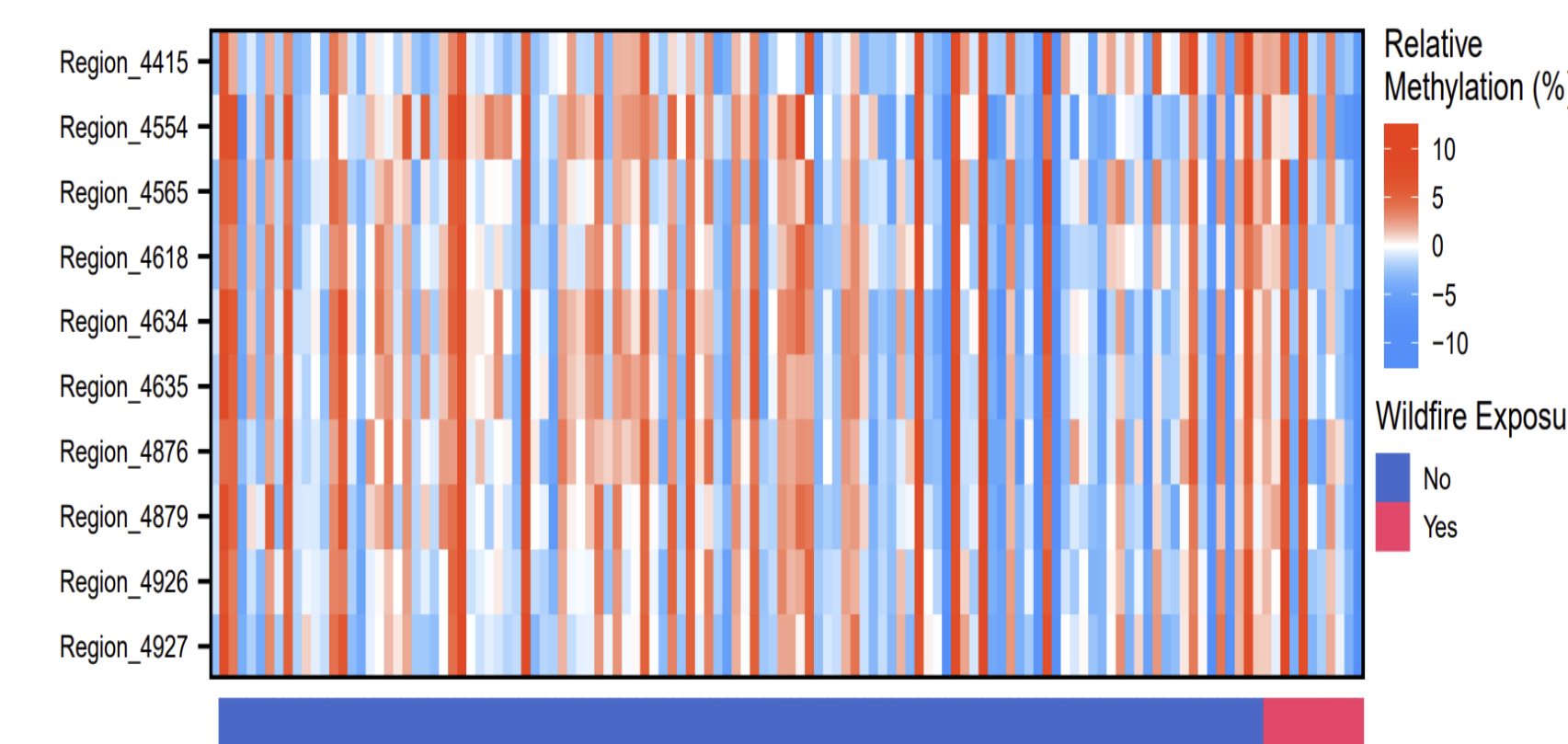


Figure 2.0

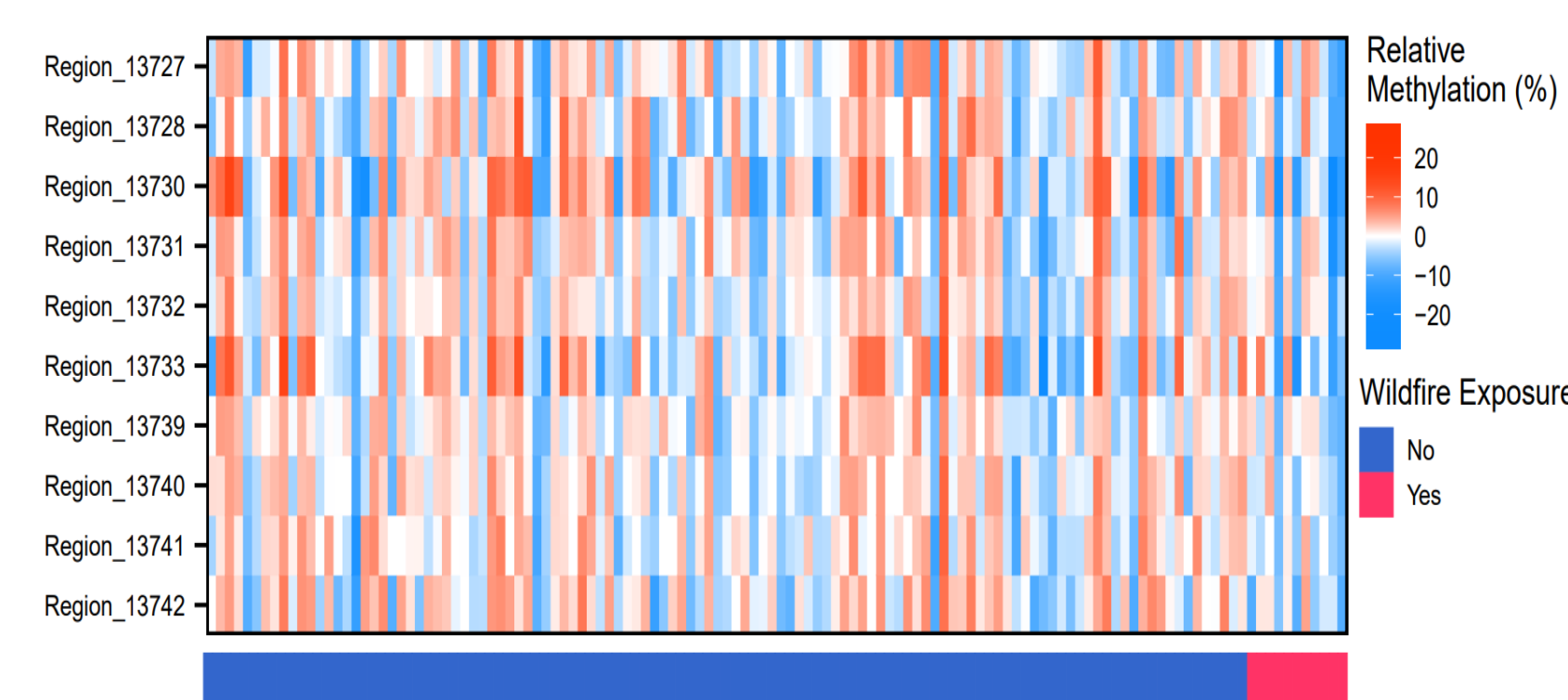


Figure 3.0

Figure 1 is a trait correlation heat map, where the modules are in colored blocks on the x-axis, while the traits are on the y-axis. We chose the highlighted trait "Wildfire Exposure" as a trait. The trait is highlighted in yellow, while the modules are circled in red. The modules were named with colors, whereas in this study they were magenta and salmon. The arrows point to these modules and the asterisks depict a significant association between methylation and wildfire exposure. **Figure 2.0** shows a methylation heatmap for the Salmon module, where the blue colored bar on the x-axis depicts the genes in regions that were not exposed to wildfire, while the red depicts certain exposure of the expectant mother to wildfire, and their respective methylation percentage on the y-axis scale. **Figure 2.1** shows the module eigenmode for the salmon module with relation to wildfire exposure, which in our experiment was on a binary scale, where "yes", a 1 used in Comethyl, meant definitive exposure to wildfire, while "no", a 0 used in Comethyl, meant no exposure to wildfire. **Figure 3.0 and Figure 3.1** show similar data produced but for the magenta module. So comparing both the modules, the salmon module had more DNA methylation compared to the magenta module when exposed to WF.

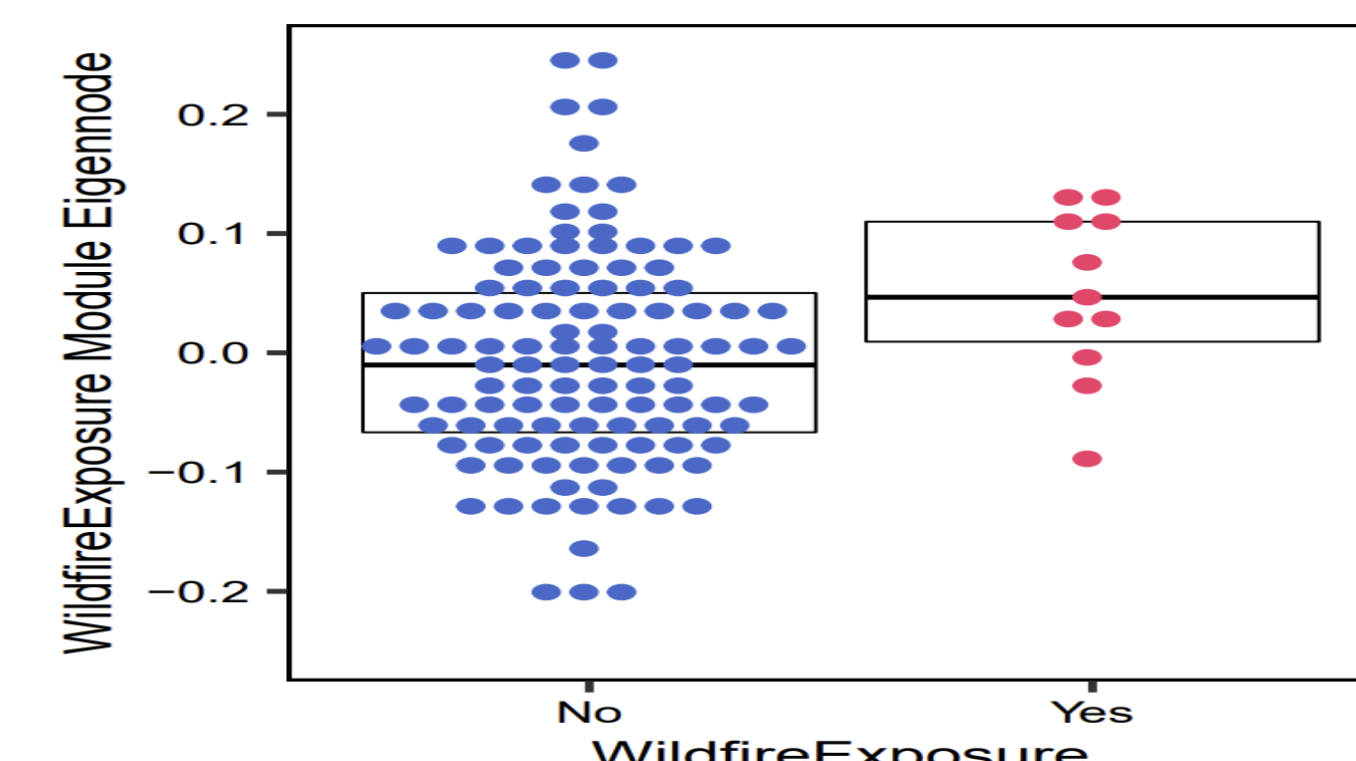


Figure 2.1

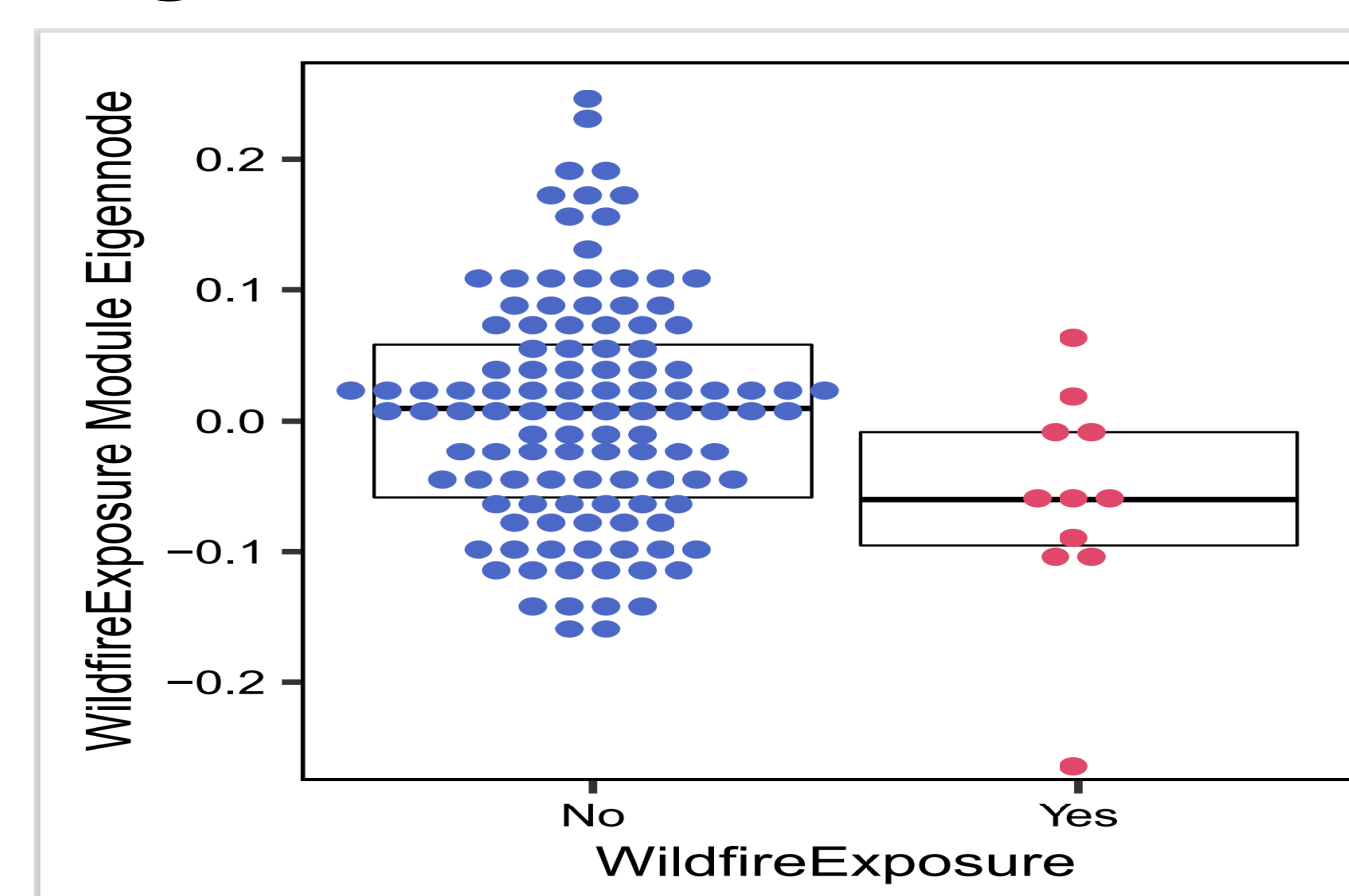


Figure 3.1

SUMMARY

- In addition to finding two gene body comethylation modules that are significantly associated with WF exposure, we found very specific genes that corresponded to the development and immune response of a human.
- From regions in the magenta and salmon modules, we found 13 and 10 genes respectively, that were responsible for being transcribed and translated into proteins that helped prevent neurodevelopment and immune disorders.
- Of those 13 genes in the magenta module, an interesting one was SMG9, which encodes a nonsense-mediated mRNA decay factor, necessary for brain development. While of the 10 genes found in the salmon module, a vital gene found was SHROOM1, which plays a factor in the development of the neural tube.
- Most of these methylated genes also comparatively correspond to persons with channelopathies⁵, which are widely found in people with ASD, bipolar disorder, and other neuropsychiatric disorders.

CONCLUSIONS

We identified two gene body comethylation modules in placenta samples that were significantly associated with prenatal wildfire exposure. Many of the genes within these modules have been found to play vital roles in immune response and neurodevelopment, based on individuals with rare mutations. If WF exposure affects the pattern of methylation of immune and neurodevelopmental genes, this could prevent optimal levels of gene expression and risk for disease. Larger sample size is needed to further test these hypotheses.

STRENGTHS & LIMITATIONS

Strengths:

- Comethyl package usability in R language
- Comethyl use reduced computational time since we used genome regions annotated as genes

Limitations:

- N= 11, low sample count
- Binary (0,1) scale for exposure. Were not able to use a scale that would utilize the intensity of exposure of the sample to wildfire

ACKNOWLEDGEMENTS & REFERENCES

I would like to thank the following people: Dr. Janine LaSalle, Logan Williams, Dr. Janice Enriquez, RISE-UP Staff members, and my colleagues.



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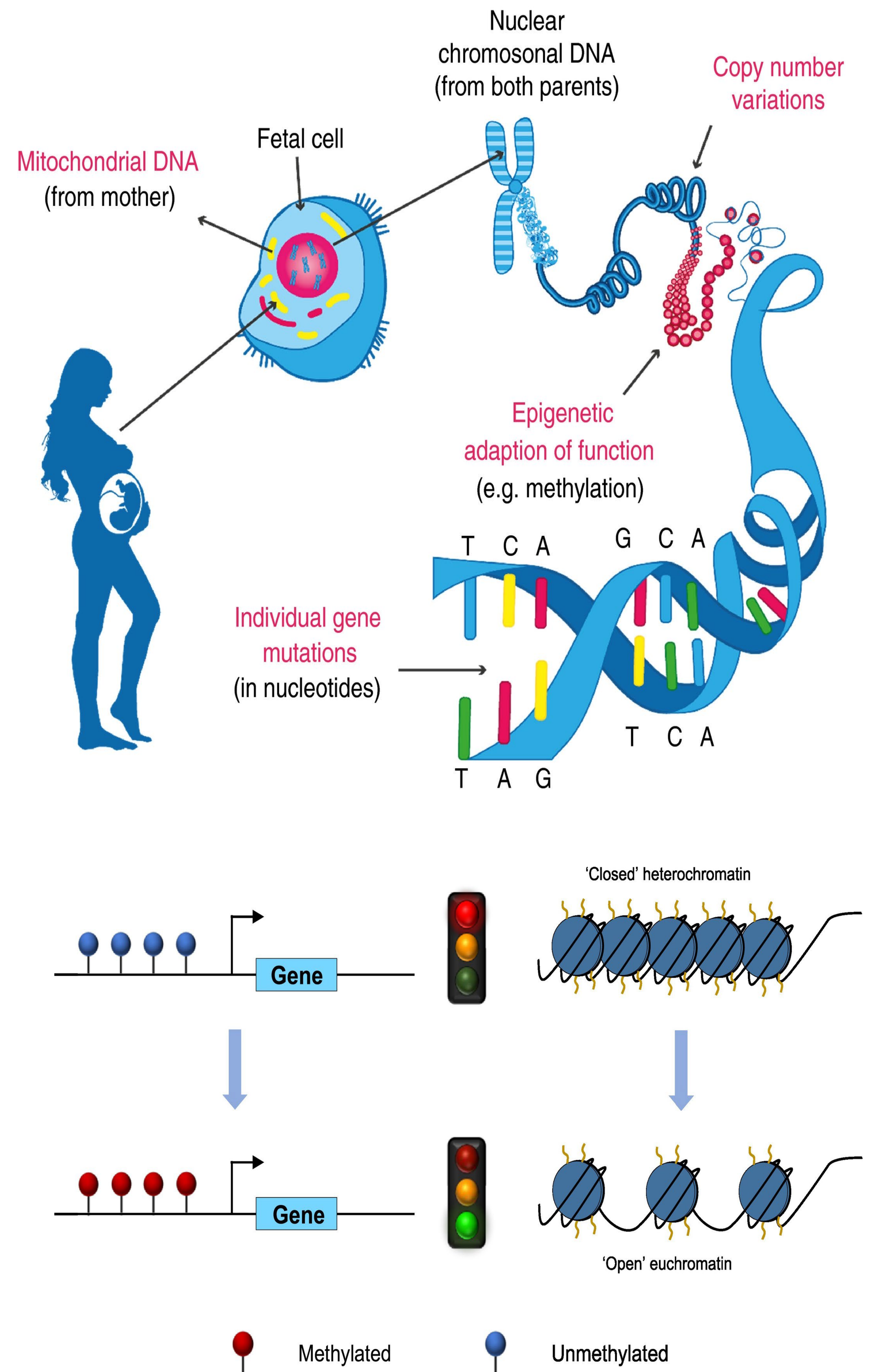
Introduction

- Evidence that exposure to air pollution increases the likelihood of **risk of autism spectrum disorder (ASD)**
- Dysregulation of fetal DNA methylation has been proposed as a mechanism for the increase in the risk of ASD diagnosis.
- In this study, we examined the **association between WF exposure and placental DNA methylation** using an R package called Comethyl.



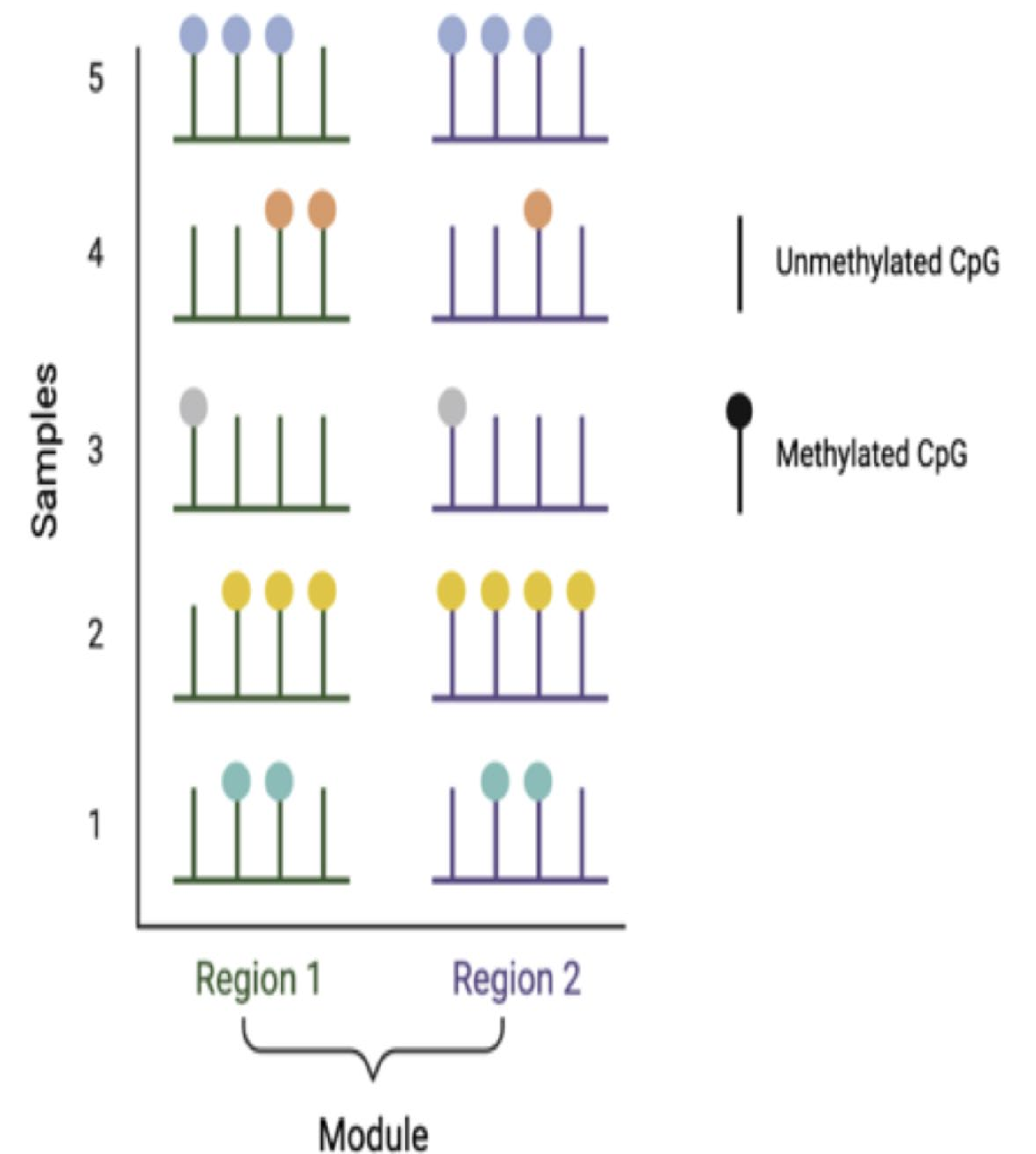
Objective

- In this study, our objective was to identify any modules of gene body comethylation in placental DNA that were significantly associated with maternal wildfire exposure.
- If such modules were identified, we would investigate their membership to identify potential candidate genes for the developmental effects of prenatal wildfire exposure.
- Why is DNA methylation important? Acts as a traffic stop – affects gene expression



Methods

- Comethyl, an R package, was used to identify and interpret modules of comethylated regions from whole-genome bisulfite sequencing data.
- Modules were identified and explored by using sample traits and functional enrichments were examined.
- Of the 126 samples that were used to generate comethylation modules, 11 were exposed to WF.



Results

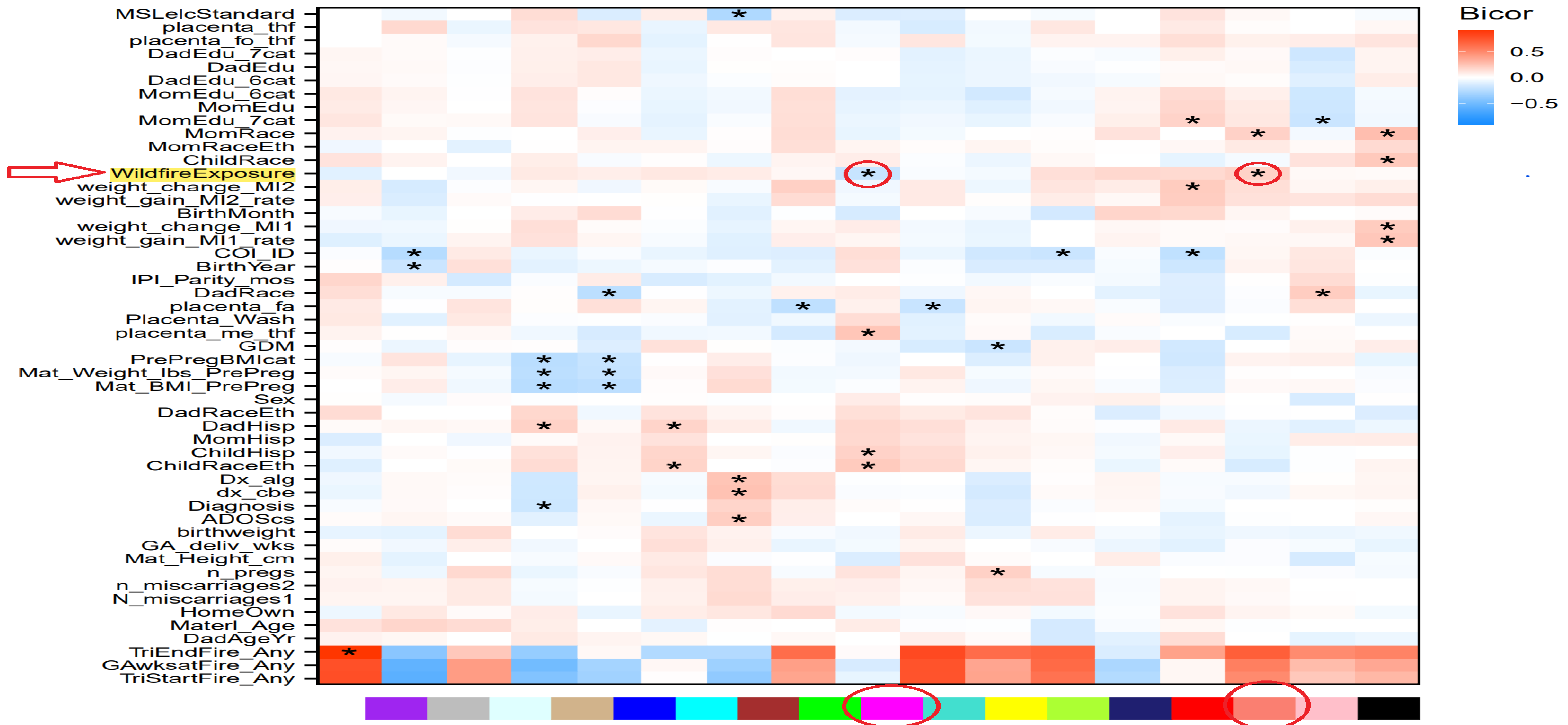


Figure 1, as mentioned in the previous slide, is a correlogram, where the modules are in colored blocks on the x-axis, while the traits are on the y-axis. We chose the highlighted trait “Wildfire Exposure” as a trait. The trait is highlighted in yellow, while the modules are circled in black. The modules were named with colors, whereas in this study they were magenta and salmon. The arrows point to these modules and the asterisks depict an association between methylation and wildfire exposure.

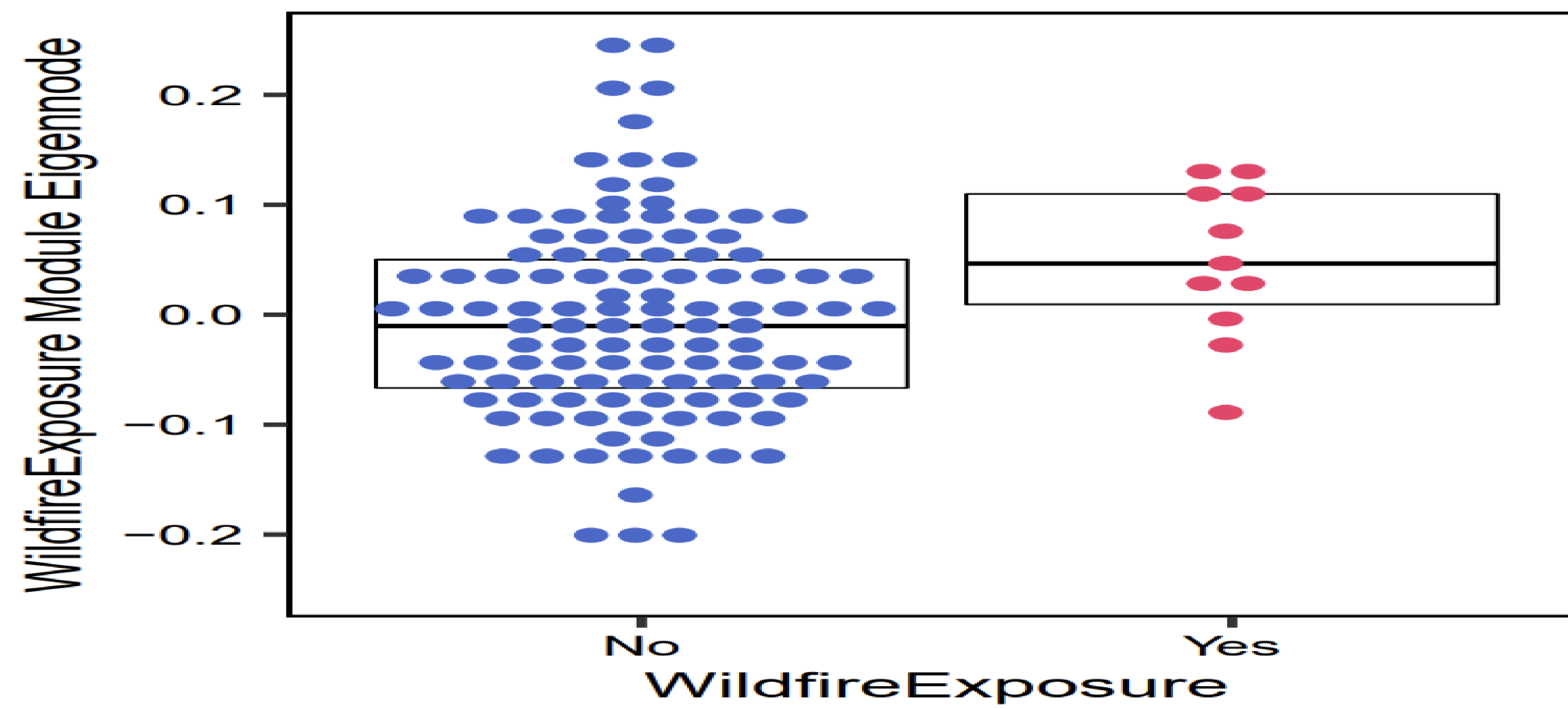


Figure 2.1
Salmon Module

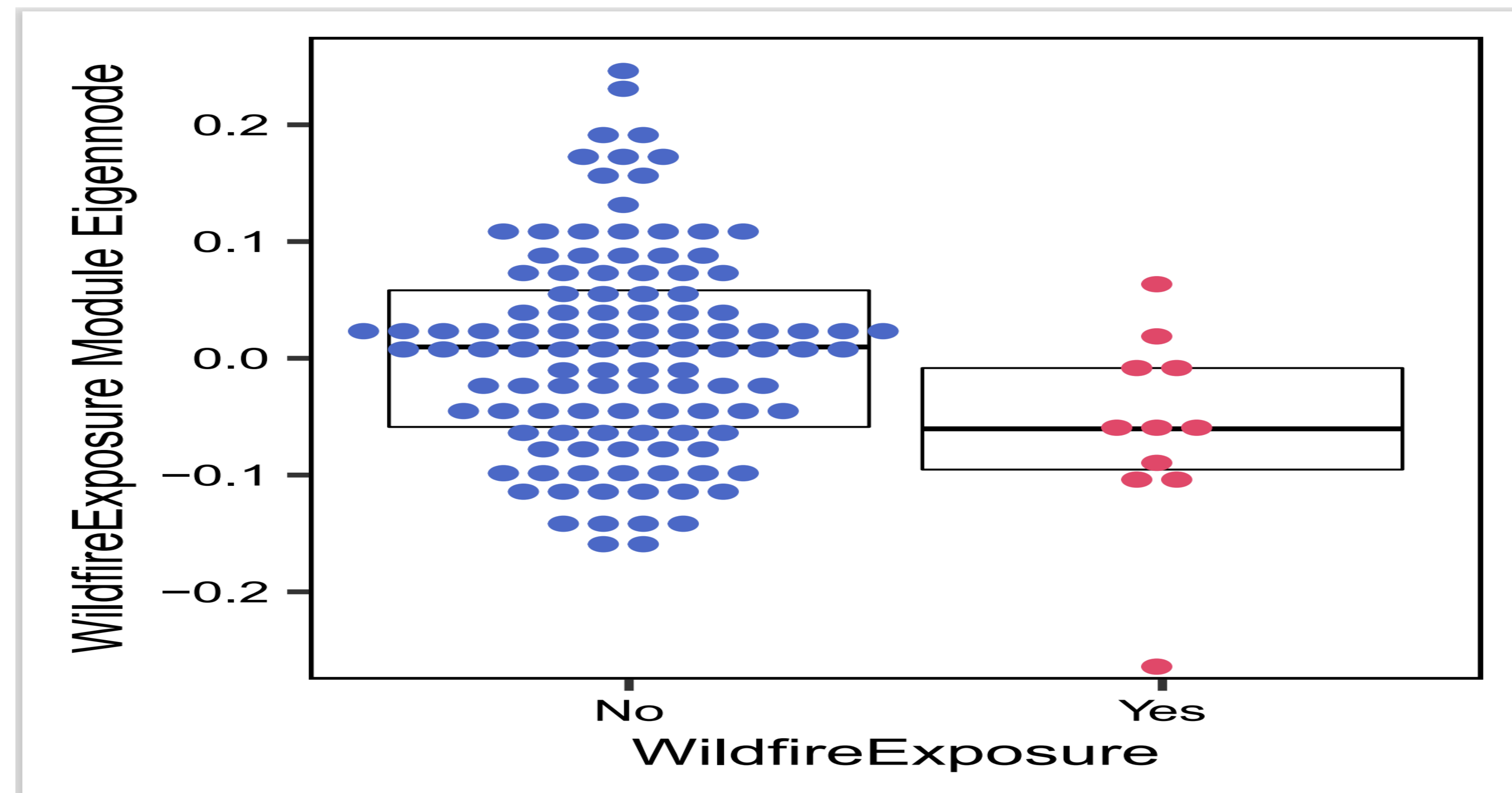


Figure 3.1
Magenta Module

- **Figures 2.1 and 3.1** show the module eigennode (general value of methylation) for the Salmon and Magenta module in relation to how less or much of the regions were methylated.
- For **figures 2.1 and 3.1**, the exposure to WF was on a binary scale, where “yes” meant definitive exposure to wildfire, while “no” meant no exposure to wildfire.
- We could say that the **salmon module had more percent methylation for those exposed to WF.**

Examples of Key Genes Contained In Modules

Magenta Module

"SMG9" - SMG9 nonsense-mediated mRNA decay factor

- This gene encodes a regulatory subunit of the SMG1 complex
- It plays a critical role in the inhibition of kinase activity
- Mutations in this gene cause a multiple congenital anomaly syndrome in human patients, characterized by a brain malformation and congenital heart disease.

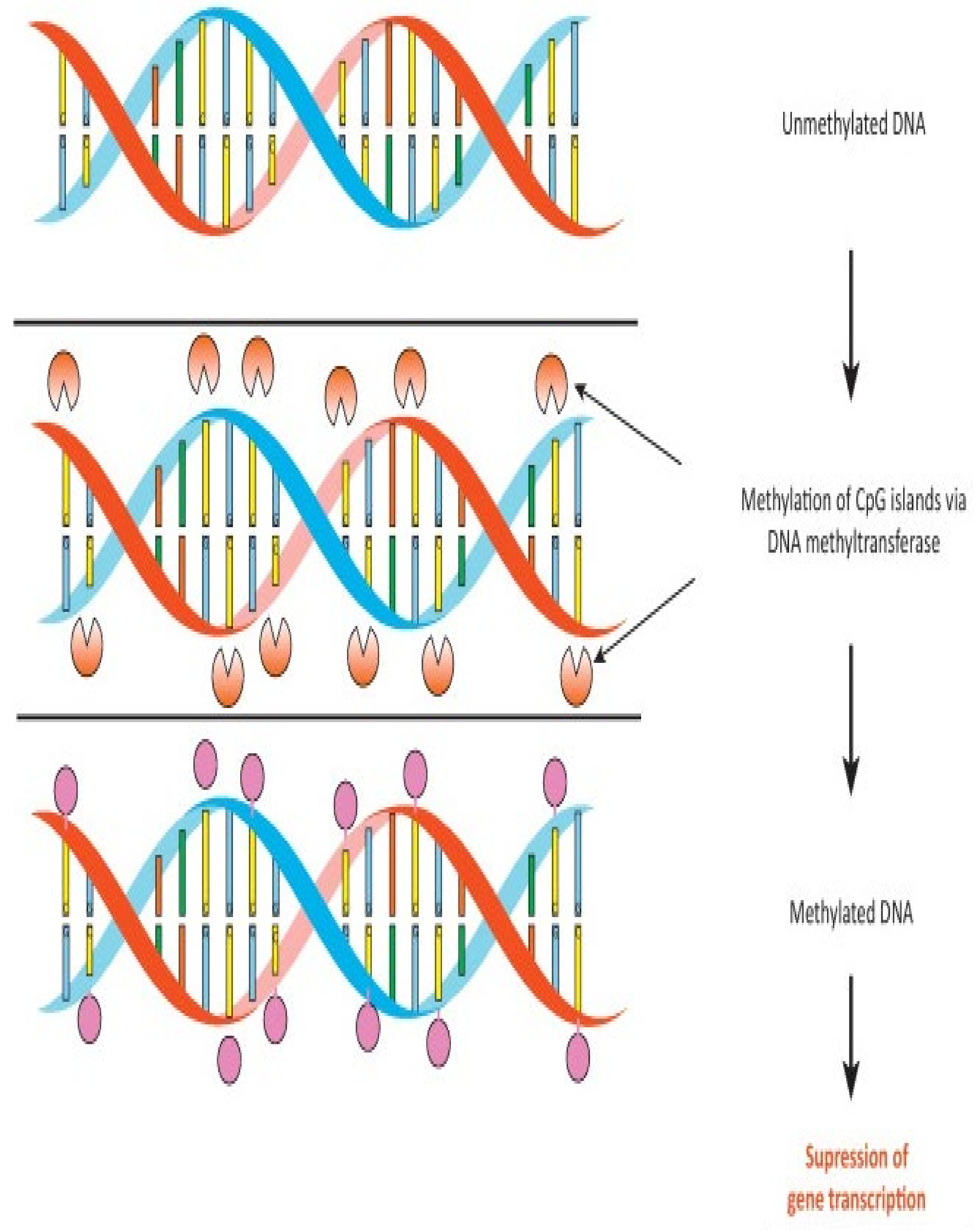
Salmon Module

"SHROOM1" - shroom family member 1

- SHROOM family members
- Encodes Acting binding protein, important in neural tube closure
- Plays diverse roles in the development of the nervous system

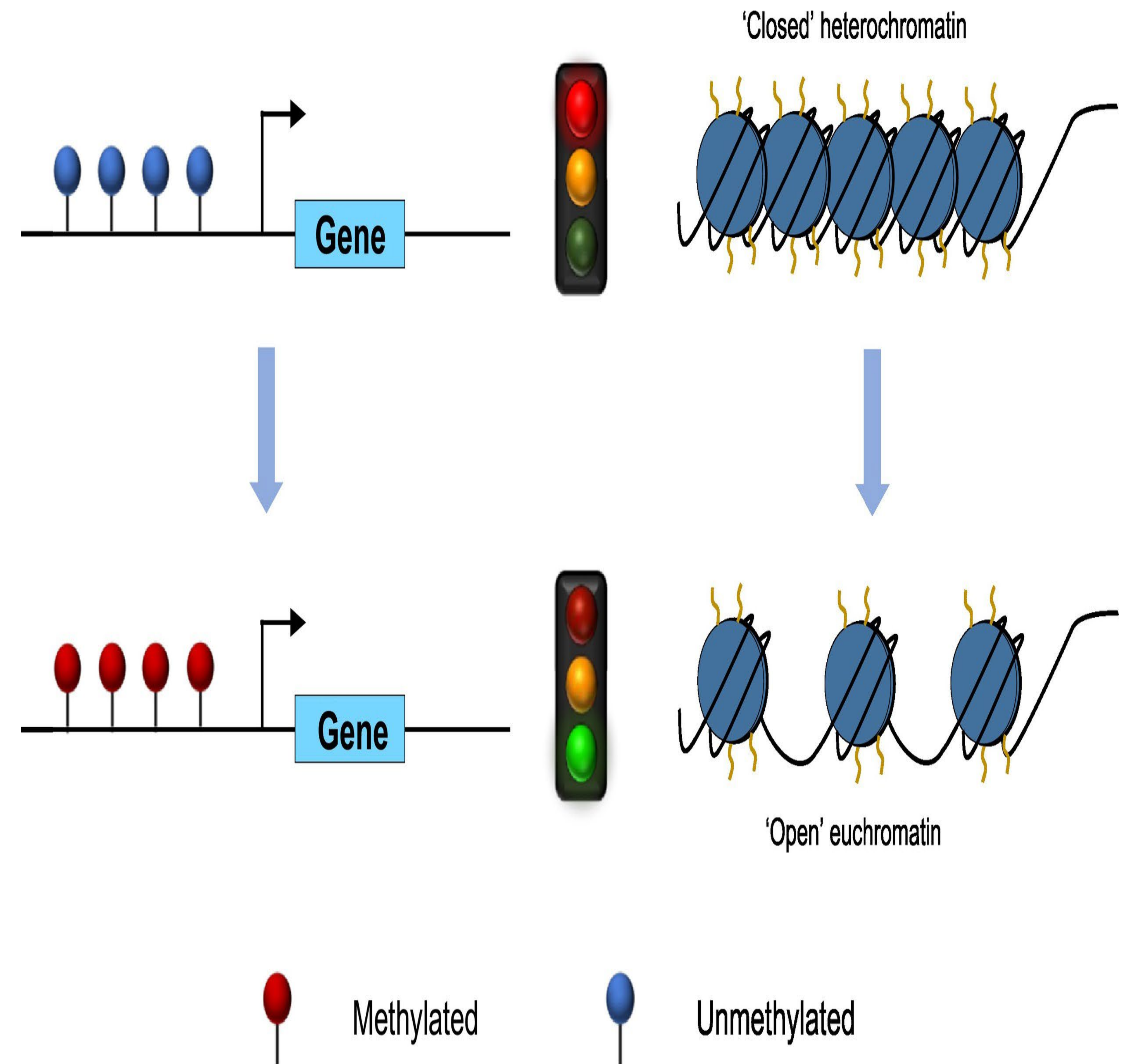
Summary

- Found very specific genes that corresponded to neurodevelopment in modules from samples exposed to WF.
- From regions in the magenta and salmon modules, we found 13 and 10 genes respectively, that were responsible for neurodevelopment
- Examples include SMG9, in the magenta module, and SHROOM1, in the salmon module.



Conclusion

- If genes are suppressed through **DNA methylation** it may result in:
 1. **Channelopathies**
 2. **Brain malformation**
 3. **Neurocognitive issues.**
- Previous research shows many of these issues are found in people **diagnosed with ASD.**
- These modules contain genes of interest for further epigenetic studies of the interaction between prenatal WF exposure and fetal neurodevelopment.



Strengths and Limitations

Strengths:

- Comethyl package usability in R language
- Comethyl use reduced computational time since we used genome regions annotated as genes

Limitations:

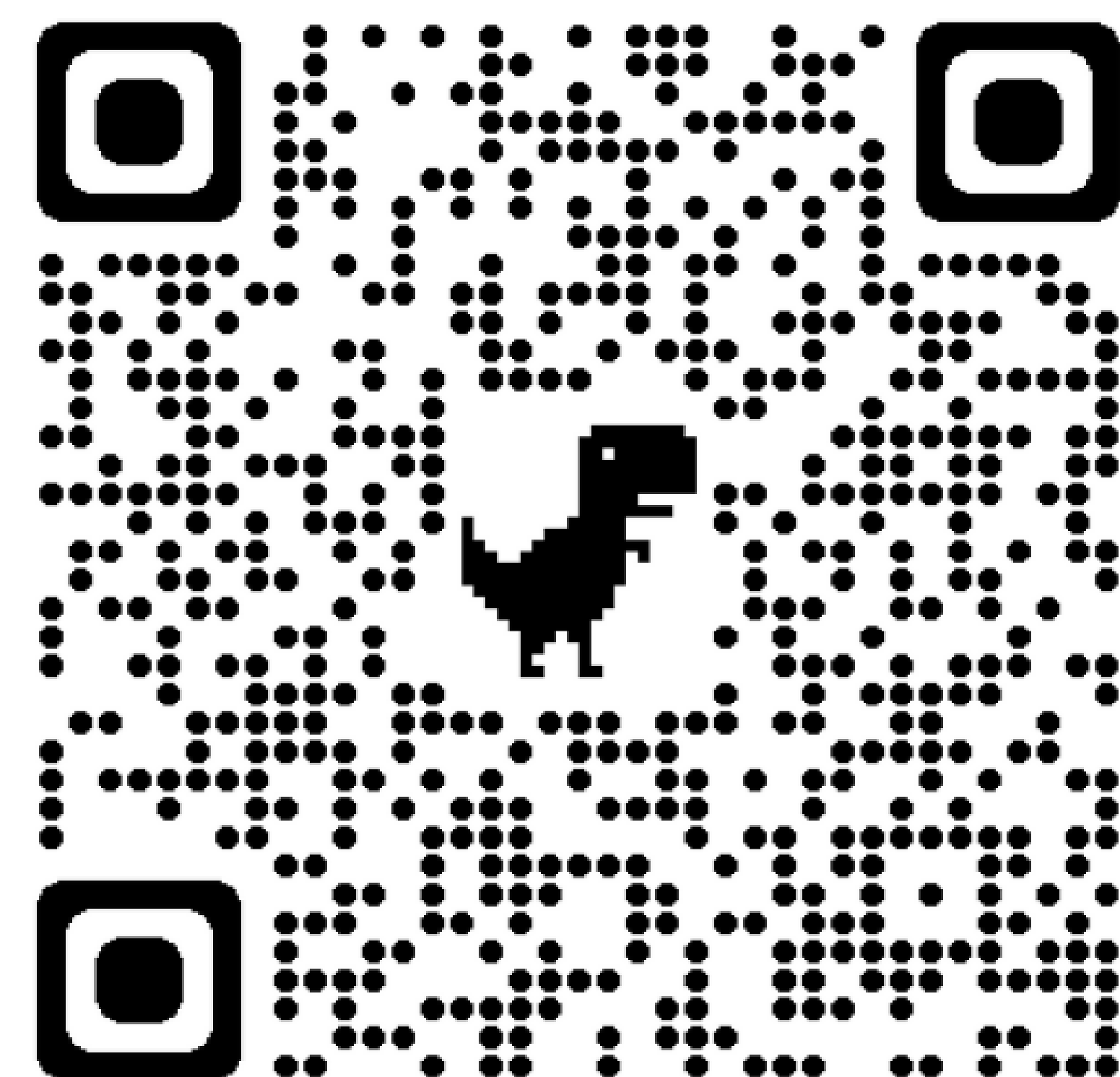
- N= 11, low sample count
- Binary (0,1) scale for exposure. Were not able to count the intensity of exposure of the sample to wildfire

Acknowledgments

I Would like to thank the following people: Dr. Janine LaSalle, my faculty mentor, Logan Williams, my student mentor, Dr. Janice Enriquez, RISE-UP Staff members, and my colleagues.

References

To access the list of sources used for this research or for the gene list, please scan the QR code on the right



Thank You