An Examination of Alzheimer Associated Gene Expression in Neuron Cells That Are Infected by Lyme Causing Bacteria

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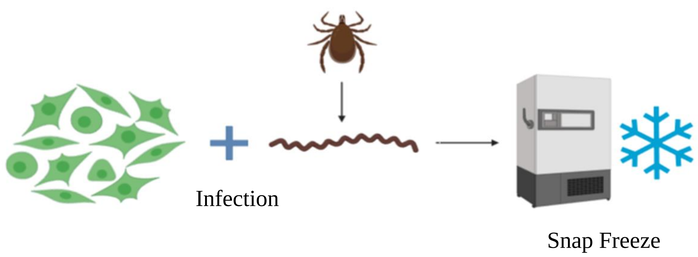
Introduction

- Alzheimer's Disease currently effects 5.8 millions of people.
- Alzheimer's Disease is the main cause of dementia and is diagnosed by biochemical lesions of mainly ß-amyloid peptide (Aß) plaques [1]
- Previous research has shown that polymicrobial infections, including *B. bugdorferi*, were found in brains of Alzheimer's patients and the DNA of *B. bugdorferi* was found in the Aß plaques in the brains of Alzheimer's patients [2,3]
- This study evaluates the expression of Alzheimer's related genes in neuron cell lines (BE2C and HEK-293) before and after infection of Lyme-causing bacteria, *B. bugdorferi*, through qPCR assays.
- The genes examined in this study are APP, PSEN1, PSEN2, APOE and p53.
- APP, PSEN1, and PSEN2 cause early-onset Alzheimer's Disease while the gene APOE is strongly linked to late-onset Alzheimer's Disease [4]

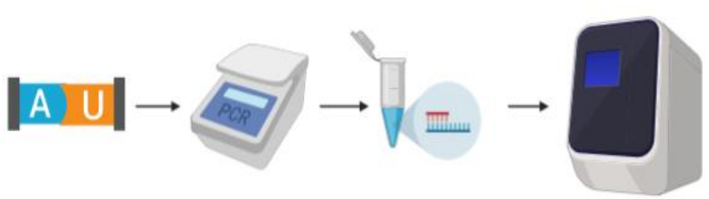
Materials and Methods

Mammalian cell culture:
BE2C neuroblastoma and neuron-like HEK-293 cells were grown at 37 degrees Celsius and 5% CO2. BE2C were cultured with EMEM-F12 media, 10% fetal bovine serum (FBS), and 1% Penicllin-Streptomycin-Glutamine (PSG). HEK-293 were cultured with DMEM, 10% FBS, and 1% PSG.

***B. bugdorferi* Infection:**
B. burgdorferi were obtained from Dr. Eva Sapi's Lab at the University of New Haven.



RNA extraction, cDNA Synthesis, and qPCR:
At 70% confluency, 1x10e6 cells were pelleted and RNA was extracted. cDNA was made using 10ng RNA, which was then used for qPCR assays.



Results

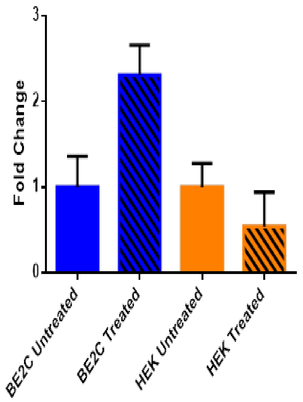


Figure 1. *qPCR fold changes of the gene APP.* Treated BE2C cells shows an up regulation while the treated HEK-293 cells show a down regulation. The error bars represent standard error of three independent data set. The p-value for treated BE2C cells was 0.0190 and for treated HEK-293 cells was 0.5034.

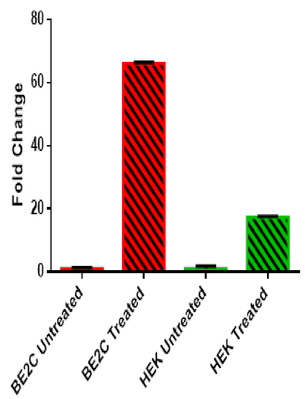


Figure 2. *qPCR fold changes of the gene PSEN1.* The fold changes for the both treated cell lines show an up regulation. The error bars represent standard error of three independent data set. The p-value for both treated cell lines were less than 0.0001.

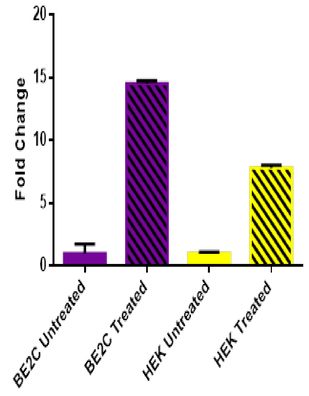


Figure 3. *qPCR fold changes of the gene PSEN2.* The fold change for the both treated cell lines show an up regulation. The error bars represent standard error of three independent data set. The p-value for both treated cell lines were less than 0.0001.

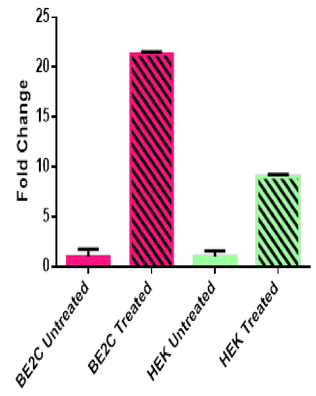


Figure 4. *qPCR fold changes of the gene APOE.* The fold change for the both treated cell lines show an up regulation. The error bars represent standard error of three independent data set. The p-value for both treated cell lines were less than 0.0001.

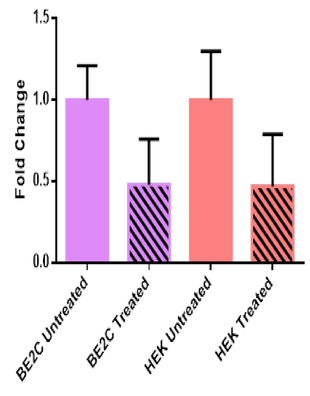


Figure 5. *qPCR fold changed of the gene p53.* The fold changes for both treated cell lines show a down regulation. The error bars represent standard error of three independent data set. The p-value for treated BE2C cells was 0.1568 and for treated HEK-293 cells was 0.2445.

Conclusions

- Statistical analysis indicates there was a significant increases in expression of Alzheimer associated genes PSEN1, PSEN2, and APOE in BE2C and HEK cells treated with Lyme causing bacteria *B. burgdorferi.*
- There was a statistically significant increase in expression in the treated BE2C cell line for the gene APP. The down regulation in the treated HEK-293 cell line for the gene APP was not significantly different, along with the down regulation in both treated cell lines for the tumor suppressor gene p53.
- **The increase in expression of all Alzheimer's related genes tested in neuronal BE2C cells shows a possible correlation between Alzheimer's Disease and Lyme Disease.**

Future Work

- Repeat experiment for reproducibility of results.
- Perform Western Blot and/or Flow Cytometry assay verify higher expression of proteins made by these genes.
- Examine other genes related to Alzheimer's Disease.
- Examine expression kinetics post infection

References

[1] Carlo Sala Frigerio, Leen Wolfs, Nicola Fattorelli, Nicola Thrupp, Iryna Voytyuk Inga Schmidt, Renzo Mancuso, Wei-Ting Chen, Maya E. Woodbury, Gyan Srivastava, Thomas Möller, Eloise Hudry, Sudeshna Das, Takaomi Saido, Eric Karran, Bradley Hyman, V. Hugh Perry, Mark Fiers, Bart De Strooper. (2019). **The Major Risk Factors for Alzheimer's Disease: Age, Sex, and Genes Modulate the Microglia Response to Aβ Plaques.** Cell Reports, Vol 27(4), 1293-1306.

[2] Diana Pisa, Ruth Alonso, Ana M. Fernandez-Fernandez, Alberta Rabano, Luis Carrasco. (2017). **Polymicrobial Infections in Brain Tissue from Alzheimer's Disease Patients.** Sci Rep, Vol 7, 5559.

[3] Alan B. MacDonald. (2006). **Plaques of Alzheimer's Disease Originate from Cysts of Borrelia burgdorferi, the Lyme Disease Spirochete.** Medical Hypotheses, Vol 67, 592-600.

[4] Mohan Giri, Man Zhang, Yang Lu. (2016). **Genes Associated with Alzheimer's Disease: An Overview and Current Status.** Clin Interv Aging, Vol 11, 665-681.

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