Caffeine Affects Alternative Splicing of the Nematode WNT Pathway Gene, *APR-1*

Helana Yacoub, Rezhin Ahmad, Rebecca Siepelt-Thiemann, Zachary Grimes

BACKGROUND

- *APR-1* is a gene located on chromosome 1
- *APR-1* has a role in endoderm cell specification and pharyngeal development (Hoier et al. 2000)
- *APR-1* was tested on nematode worms, specifically *C-elegans.*
- In low doses, caffeine has been shown to extend life-span in the soil nematode.
- Caffeine has been shown to induce alternative splicing of genes in the WNT signaling pathway.
- For the purpose of this experiment *C-elegans* were treated with 380 mg of caffeine.

HYPOTHESIS

- The *APR-1* gene will exhibit alternative splicing in the presence of caffeine.

MATERIALS AND METHODS

- The National Center for Biotechnology Information was used to research the structure of *APR-1* gene and identify the regions where alternative splicing may happen. The included exons are 2,4 and exon 3 was excluded.
- mRNA was isolated from the worms that were grown.
- Using reverse transcription, cDNA was gathered from control and experimental sample.
- Two tests were performed using gradient PCR and PCR. Primers designed especially for the precise region were used to amplify the target. An agarose gel electrophoresis was used to analyze the optimal annealing temperature of the primers
- electrophoresis was used to analyze the samples.
- Using USCS genome browser, expasy, and interproscan sequences and translations were performed *in silico*.

RESULTS

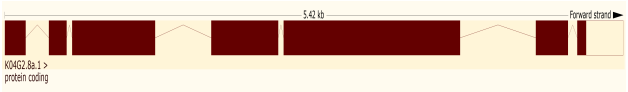

Figure 1. Gene map of *APR-1* gene showing exons and introns

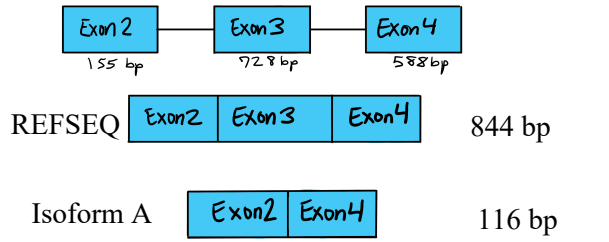

Figure 2. Predicted isoforms and expected RT-PCR sizes.

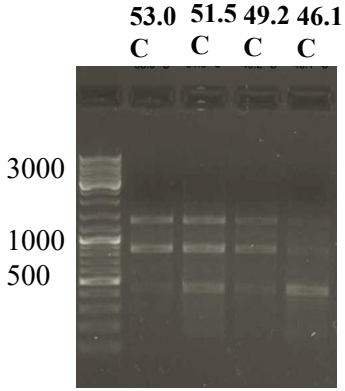

Figure 3. Reverse-Transcription-Polymerase Chain Reaction (RT-PCR) Results Indicate *APR-1* Primers Anneal Best at 53.0°C.

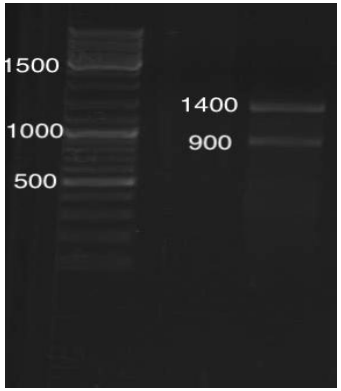

Figure 4. PCR was analyzed at 100 V for 30 minutes results are indicted in the above electrophoresis

CONCLUSION

- The control sample was damaged, but the results showed that alternative splicing occurred in the experimental sample.
- The domain search showed no result. For *APR-1*
- The gradient PCR analysis shows that alternative splicing occurred in the experimental sample at the optimal annealing temperature of 53.0 °.

FUTURE DIRECTION

- The original hypothesis depended on the amount of caffeine the average person consumes and the effect it would have on *APR-1*
- If we were to continue experimenting, we would repeat the experiment again before experimenting on a larger animal.
- After successfully repeating the experiment a few times on animals like mice, we would eventually introduce the *APC* gene to human DNA in order to see the potential affects caffeine could have on colon cancer.

REFRENCES

- Gan Y, Wu J, Zhang S, Li L, Cao S, Mkandawire N, Ji K, Herath C, Gao C, Xu H, et al. Association of coffee consumption with risk of colorectal cancer: a meta-analysis of prospective cohort studies. Oncotarget. 2017 Mar 21 [accessed 2020 Apr 10]. https://www.ncbi.nlm.nih.gov/pubmed/27078843
- Groessl EJ, Allison MA, Larson JC, Ho SB, Snetslaar LG, Lane DS, Tharp KM, Stefanick ML. Coffee Consumption and the Incidence of Colorectal Cancer in Women. Journal of cancer epidemiology. 2016 [accessed 2020 Apr 10]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4864536/
- Hoier EF, Mohler WA, Kim SK, Hajnal A. The Caenorhabditis elegans APC-related gene apr-1 is required for epithelial cell migration and Hox gene expression. Genes & development. 2000 Apr 1 [accessed 2020 Apr 10]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC316495/