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

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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 lifespan 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 °C.

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.

REFERENCES

  https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4864536/
  https://www.ncbi.nlm.nih.gov/pmc/articles/PMC316495/