Effect on Hypo/hyper-methylation Rates on Genome Regions CDKN1A, BMAL1, NR1C3, and PNPLA3 with CBG Implementation Utilizing a Mice Model with MCD Diet to Induce NASH

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Background

- Non-alcoholic fatty liver disease (**NAFLD**) is the most prominent liver disease in the world
- Non-alcoholic steatohepatitis (**NASH**) is defined when inflammation is associated with hepatic steatosis
- Methionine/choline deficient (**MCD**) diet induced NASH in mice model
- Methylation is the addition of a methyl group (CH_3) at CpG dinucleotide sites (DMNTs)
- DNA methylation = gene silencer (hypermethylation associated with NASH pathology)
- Cannabigerol (**CBG**) = a non-psychotropic cannabinoid
- Experimental conditions = MCD, MCD Low CBG, MCD High CBG, Control, Control Low CBG, and Control High CBG

Research question: what is the hypo/hypermethylation effect on promoter regions of CDKN1A, BMAL1, NR1C3, and PNPLA3 genes with CBG implementation utilizing a mouse model with MCD diet to induce NASH?

Hypothesis 1: MCD diet will hypermethylate the four genes compared to the control diet

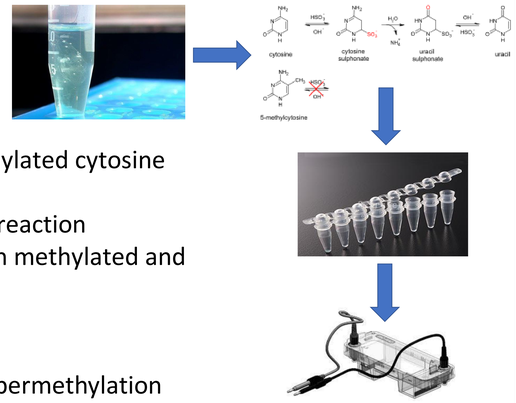
Hypothesis 2: CBG implementation will hypomethylate the four genes

Gene NAFLD pathology relation

1. **PNPLA3:** Encodes lipase protein
   - Catabolic activity towards triglycerides
2. **CDKN1A**: Encodes p21 protein
   - Transcriptional p53 target, cell cycle inhibitor, and cellular senescence
3. **BMAL1:** Encodes circadian clock protein
   - Fat storage/utilization and adipocyte differentiation
4. **NR1C3:** Encodes PPAR-γ protein
   - Lipid metabolism and adipocyte differentiation

Methods

1. **DNA extraction**
   - DNA extracted from liver tissue
2. **Bisulfite treatment**
   - Distinguish the methylated and unmethylated cytosine
3. **MSP**
   - Methylation-specific polymerase chain reaction
   - Amplify quantity of the four genes (both methylated and unmethylated)
4. **Gel-electrophoresis**
   - DNA separation
   - Indicated either hypomethylation or hypermethylation



Methylation-specific PCR (MSP) – PNPLA3

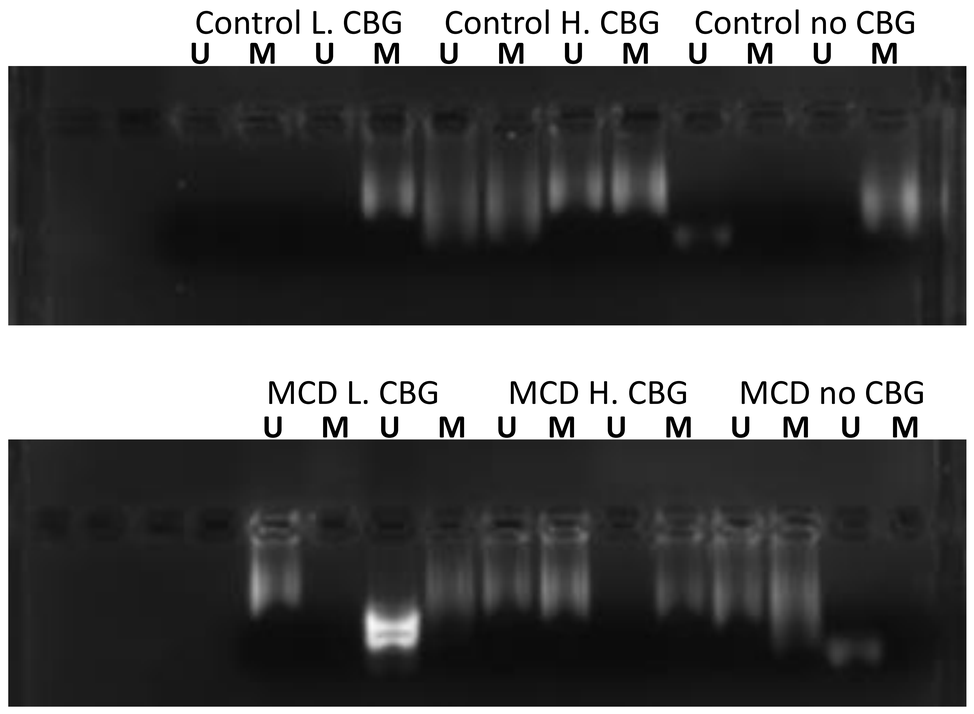


Figure 1: Gel-electrophoresis data for PNPLA3 gene. Each experimental condition had two mouse samples. Each mouse sample was PCR amplified for both methylated and unmethylated version of PNPLA3 gene. The "M" signifies the methylated sample, and the "U" signifies the unmethylated sample.

Results & Conclusion

- PNPLA3 gene: **Hypo**methylation
- CDKN1A gene: NA
- BMAL1 gene: NA
- NR1C3 gene: NA

Hypothesis 1: For PNPLA3, this hypothesis is rejected

Hypothesis 2: For PNPLA3, this hypothesis is accepted (High CBG only)

- Only had time to collect data for PNPLA3 gene
- In the groups with no CBG, MCD diet hypomethylated PNPLA3 gene compared to Control diet
- In the groups with High CBG, the PNPLA3 gene was hypomethylated (not Low CBG groups)

Future Direction

- Gather data on CDKN1A, BMAL1, and NR1C3
- Investigate the role of cannabinoids, such as CBG, in alleviating the damage caused by MCD diet
- Explore whether CBG could be a potential therapeutic approach for NAFLD patients

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Selected references: Eslam, M., Valenti, L., & Romeo, S. (2018). Genetics and epigenetics of NAFLD and NASH: Clinical impact. *Journal of Hepatology,* 68(2), 268-279. https://doi.org/doi: 10.1016/j.jhep.2017.09.003. Tryndyak, V., Han, T., Fuscoe, J., Ross, S., Beland, F. & Pogribny, I. (2016). Status of hepatic DNA methylome predetermines and modulates the severity of non-alcoholic fatty liver injury in mice. *BMC Genomics,* 17, 298. https://doi.org/10.1186/s12864-016-2617-2. Trépo, E., Romeo, S., Zucman-Rossi, J., & Nahon, P. (2016). PNPLA3 gene in liver diseases. *Journal of Hepatology,* 65(2), 399-412. https://doi.org/10.1016/j.jhep. 2016.03.011.