DNA Methylation and Genetic Variation of the Membrane-bound Catechol-O-methyltransferase (*MB-COMT*) Gene in Saliva of Healthy Individuals

Maria-Lucia Delgado[1] and Toni-Ann Bravo[2]

Mentors: Lissette Delgado-Cruzata, PhD, MPH[2], Nataša Vučinić PhD[3], and Snežana Smederevac, PhD[3]

[1]Mount Holyoke College, MA [2]John Jay College, CUNY, NY [3]Novi Sad University, Serbia

ABSTRACT

One of the enzymes involved in the degradation of the neurotransmitter dopamine is membrane-bound catechol-O-methyltransferase (MB-COMT). Analysis of the Val[158]Met single nucleotide polymorphism (SNP) of MB-COMT is important to its activity, and has shown that the Val/Val (GG) variant is associated with higher dopamine degradation and substance use. *MB-COMT* is epigenetically regulated by promoter region DNA methylation. Previous research showed that decreased blood DNA methylation, leading to increased gene expression, in carriers of the Met/Met (AA) allele was associated with less likelihood of substance use. Here, we study the association of DNA methylation in the promoter of *MB-COMT* and Val[158]Met SNP genetic variation in saliva samples of 85 healthy individuals. DNA was extracted from saliva, bisulfite converted and *MB-COMT* promoter DNA methylation determined by pyrosequencing. Taqman assays were used to assess carrier status. We used t-test and ANOVA when appropriate to determine differences in DNA methylation between genotypes. We found that saliva DNA methylation was not significantly different between the GG, AG and AA genotypes (4.6 ± 4.7%, 4.3 ± 3.3% and 4.2±2.4% for the AA, AG and GG genotypes respectively (p=0.88)). Our preliminary results suggest that there is no correlation in saliva between carrier status and DNA methylation in the *MB-COMT* promoter region. We will continue our studies investigating *COMT* and its connection to substance use to uncover further knowledge on our own genome and ultimately, mental illness.

INTRODUCTION

- Membrane bound Catechol-O-methyltransferase, *MB-COMT*, is a gene that codes for an enzyme tasked with the degradation of dopamine, a neurotransmitter (Figure 1)

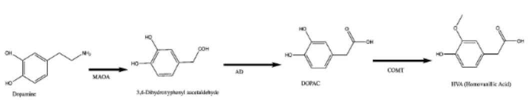


Figure 1. Dopamine Degradation Mechanism[10]

- *MB-COMT* is found in high concentrations within the prefrontal cortex of the brain determines personality, behavior inhibition, thinking, short term memory, and emotion regulation.[1,2]
- A A/G single nucleotide polymorphism in *MB-COMT* results in a Val[158]Met substitution. Three genotypes exist at this loci: G/G (Val/Val), A/G (Met/Val), and A/A (Met/Met)[8,9]
- The Val variant has a higher rate of dopamine degradation and studies have shown that the Met/Met variant leads to less likelihood of addiction than the Val/Val [1,3]

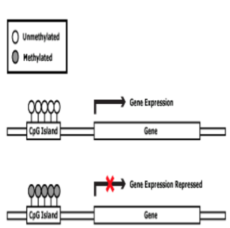


Figure 2. Gene silencing via DNA methylation in CpG Island. Modified from Diagenode Epigenetic Solutions Bisulfite Conversions Kit Visual.[5]

- *MB-COMT* is known to be epigenetically regulated via DNA methylation of its promoter region[7]
- Lack of DNA methylation at a promoter leads to transcription factors binding and initiation of transcription
- Promoter DNA methylation results in the transcription factor not being allowed to bind and inhibition of transcription[12]

The objective of this research is to determine whether DNA methylation in the *MB-COMT* promoter region varies by genotype of the Val[158]Met polymorphism.

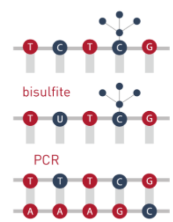
METHODS

Saliva Samples
85 healthy individuals

↓

DNA Extraction

DNA methylation Analysis → **Bisulfite Conversion & PCR Amplification**



↓

MB-COMT Pyrosequencing

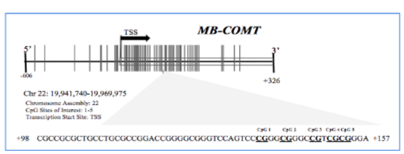


Figure 3. Map of the *MB-COMT* promoter and locations of the CpG sites measured in this experiment. CpG sites are labeled.

Genotyping → **Taqman Genotyping Assay**

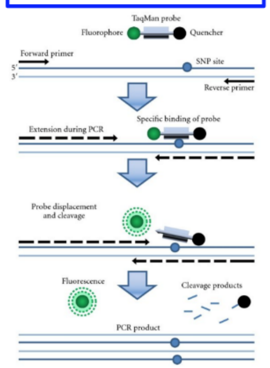


Figure 4. Taqman Assay. Modified from .[6]

↓

Statistical Analysis: *t*-test, ANOVA

Acknowledgements

Thank you to the study participants for their contribution. Another thank you to the CUNY Research Foundation and the College Now program for their support in completing this project.

Thank you to The Lynk Universal Application Funding provided by Mount Holyoke College.

RESULTS

Table 1: Study Population depicted by MB-COMT Val[158]Met genotype.

Carrier Status	Number of Individuals (n)	Percentage (%)
AG	40	47%
AA	30	35%
GG	15	18%

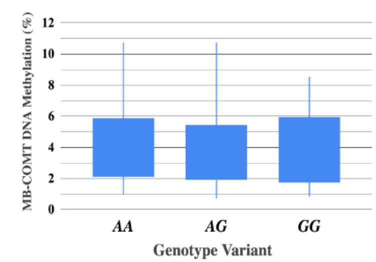


Figure 5. Average DNA methylation percentage for carriers of Val[158]Met genotypes. Box plot of DNA methylation percentages at the promoter region of *MB-COMT* for each genotype.

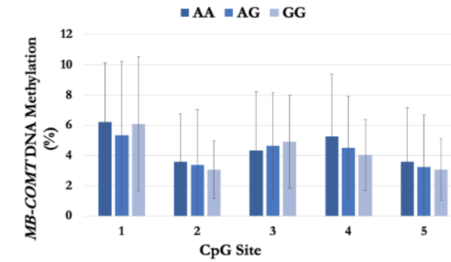


Figure 6. Percent DNA methylation at individual CpG sites by carrier status. DNA methylation percentage of each CpG site (depicted in Figure 2) measured in the promoter of *MB-COMT*

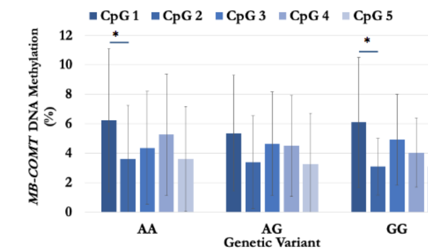


Figure 7. *MB-COMT* DNA methylation percentage among sites for individual genotypic variants. Average percentage of DNA methylation for each of the five CpG sites for each genotype. *P*-values ≤ 0.05 are indicated.

CONCLUSIONS

Summary of Results:

- We found that there was no difference in the levels of *MB-COMT* DNA methylation in saliva between carriers of the *AA, AG* and *GG* genotypes (ANOVA p=0.88) in this sample
  - Average *MB-COMT* DNA methylation was 4.6±3.6%, 4.3±3.3% and 4.2±2.5% for *AA, AG* and *GG* genotypes, respectively (Figure 5)
  - No differences were identified by individual CpG site either between different genotypes (Figure 6)
- In the overall sample, CpG1 had a higher DNA methylation percentage than the other sites and there was a significant difference across CpG sites (ANOVA test p=0.00026)
  - For individual genotypes, CpG1 was consistently statistically significantly higher than CpG2 and CpG5 (all p-values<0.01, t-test) (Figure 7)

Conclusions:

- Our findings suggest that healthy individuals have no difference in *MB-COMT* DNA methylation levels in saliva by different *MB-COMT* genotypes in the Val[158]Met polymorphism
- This could mean that DNA methylation and genetic variation at Val/Met at position 158 are not interacting to promote addictive behaviors but this needs to be further investigated.

Next Steps:

- In future research we would investigate a larger sample size to confirm these initial findings and correlate these biological markers to behavioral information of the participants

References

1. Chen, J., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M., Melhem, S., . . . Weinberger, D. R. (2004). Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (*COMT*): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain. *The American Journal of Human Genetics,75*(5), 807-821. doi:10.1086/425589
2. Abdolmaleky, H. M., Cheng, K., Faraone, S. V., Wilcox, M., Glatt, S. J., Gao, F., . . . Thiagalingam, S. (2006). Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Human Molecular Genetics,15*(21), 3132-3145. doi:10.1093/hmg/ddl253
3. Mowry, C., Pimentel, A., Sparks, E., & Hanlon, B. (2013). Materials characterization activities for "Take Our Sons&Daughters to Work Day" 2013. doi:10.2172/1096449
4. (n.d.). Retrieved from http://missinglink.ucsf.edu/lm/genes_and_genomes/methylation.html
5. Diagenode.com. (n.d.). Retrieved from https://www.diagenode.com/en/applications/dna-bisulfite-conversion
6. TaqMan SNP genotyping. (2017, February 13). Retrieved from http://www.applied-maths.com/applications/taqman-based-snp-genotyping
7. Roth, T. L., Lubin, F. D., Sodhi, M., & Kleinman, J. E. (2009). Epigenetic mechanisms in schizophrenia. Biochimica Et Biophysica Acta (BBA) - General Subjects,1790(9), 869-877. doi:10.1016/j.bbagen.2009.06.009
8. *COMT* catechol-O-methyltransferase [Homo sapiens (human)] - Gene - NCBI. (n.d.). Retrieved from https://www.ncbi.nlm.nih.gov/gene/1312
9. Rakvåg, T. T., Ross, J. R., Sato, H., Skorpen, F., Kaasa, S., & Klepstad, P. (2008). Genetic Variation in the Catechol-O-Methyltransferase (COMT) Gene and Morphine Requirements in Cancer Patients with Pain. *Molecular Pain,4*. doi:10.1186/1744-8069-4-64
10. ChemDraw Ultra 9.0. CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA 02140. www. cambridgesoft.com
11. Jin, B., Li, Y., & Robertson, K. D. (2011). DNA Methylation: Superior or Subordinate in the Epigenetic Hierarchy? *Genes & Cancer,2*(6), 607-617. doi:10.1177/1947601910393957
12. R. A., & C. H. (1991). DNA methylation and gene expression. *Annual Review of Microbiology,45*(1). doi:10.1146/micro.1991.45.issue-1
13. FitAmp Blood and Cultured Cell DNA Extraction Kit (50 samples). (n.d.). Retrieved from https://www.epigentek.com/catalog/fitamp-blood-and-cultured-cell-dna-extraction-kit-p-1185.html
14. How Does Bisulfite Pyrosequencing Work? (2017, December 04). Retrieved from https://toptipbio.com/bisulfite-pyrosequencing/