Modulating Alternative Splicing of MAO-A Gene Transcript

Alyssa Biondo
Fordham University
Bronx, New York

Abstract:

Monoamine oxidase A (MAO-A) or notoriously known as the "warrior gene", has recently contributed to the notion that genes can influence an individual's behavior. Studies have indicated that differential expression of MAO-A has been correlated with various psychiatric diseases and developmental disorders. The MAO-A disease related studies focused predominantly on males, due to the fact they only carry one copy of the X chromosome and would be more likely to show signs of altered behavior if they carried mutations in the gene. The under expression of MAO-A, or the presence of a variant allele which encodes for a less functional active enzyme, is believed to be associated with autism [2,6], antisocial disorder [2,4], ADHD [6,2], and aggressive behavior [6]. The over expression of MAO-A is associated with major depression disorder [1,4]. MAO inhibitors (MAOi) are potent treatments for MAO-A overexpression, however there are potentially dangerous side effects with their use [8]. HepG2 cells were treated with chemical compounds to modulate the alternative splicing in MAO-A transcripts for 24 hours. Alternative splicing events were observed in cells treated with compound #135. Variant spliced transcripts can help us understand MAO-A's biological significance in monoamine metabolism, impact on behavior and psychiatric disorders, as well as investigate its's 3D structure. Additionally, treatments that alter splicing of the MAO-A gene transcript could be potentially used as effective antipsychotics.

Introduction:

Monoamine oxidase A is encoded by a gene MAO-A located on the short arm of chromosome X, and contains 16 exons and 15 introns (Fig 1).

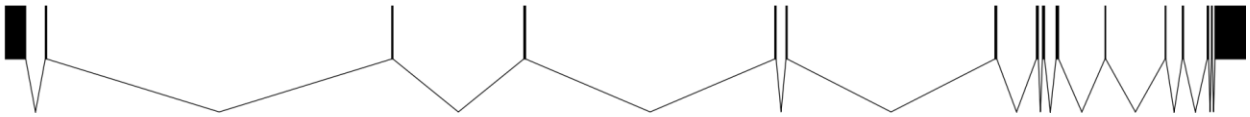

Figure 1. A schematic of the human *MAO-A* gene drawn to scale, including16 exons and 15 introns.

MAO-A is a flavoenzyme that is tethered to the outer membrane of the mitochondria and plays a vital role in the deamination of dietary monoamines and neurotransmitters [2,3].

The dimensions of MAO-A protein structure and mechanism involved in substrate deamination are not fully understood, however earlier studies have found that MAO-A contains an active site for FAD prosthetic group, which plays a crucial role in oxidative reactions. Mutagenesis studies have shown that two cysteines residues (cys372 and 406) are essentially for MAO-A substrate catalysis [8,9]. These amino acids may potentially function to bind the FAD cofactor. The same study noted that FAD may not only be an essential catalytic driver in MAO-A function, but more importantly functions to orientate the catalytic core of the enzyme [11]. After MAO-A has deaminated a substrate, the amine groups are safely removed and fed into the urea cycle as ammonia, or the backbones are subsequently utilized in protein synthesis.

MAO-A is responsible for the deamination of neurotransmitters, such as serotonin, dopamine, and epinephrine, and norepinephrine. These monoamines are vital in regulating human behavior, mood, and hormone release, therefore it is not surprising that MAO-A expression has been used as a diagnostic marker for psychiatric diseases. Over expression of the MAO-A gene have been associated with major depression disorder, sleep disturbances and suicidal tendencies [1,5]. Individuals expressing low MAO-A or less proficient forms of the enzyme are at higher risk for ADHD, autism, antisocial disorder, and aggressive/impulsive tendencies [1]. MAO inhibitors (MAOi) function to decrease MAO-A activity, thereby increasing the availability of neurotransmitters. However, there have been dangerous risks associated with the use of these drugs such as hypertensive crisis, withdrawal syndrome, and fatal drug interactions [8]. Recent studies have found that gene mutations and environmental factors equally impact individuals with MAO-A associated diseases. For instance, individuals with decreased expression of MAO-A who smoked were more inclined to aggressive behavior compared to those that had the same mutation but don't smoke [6]. There is reason to believe that chemicals in cigarette smoke can induce conformational changes to the monoamine oxidase protein by interacting with reactive amine groups, and thereby decreasing MAO-A protein activity [6].

The goal of this experiment was to see if chemical compounds can be used to manipulate MAO-A expression, or induce alternative splicing events. Other compounds can be used to induce changes in gene expression and alternative splicing of MAO-A transcript as potential therapies in place of MAO inhibitors (MAOi) which are commonly used as antidepressants with dangerous side effects. The splice variants can also be used in determining essential protein domains or used as diagnostic tools for psychiatric diseases.

Material and Methods:

Cell Culture:

HepG2, liver carcinoma cells, were treated with a variety of compounds and incubated at 37°for 24 hours. Untreated HepG2 were used as a control group and were handled in the same environmental conditions.

RNA Purification:

After RNA extraction was performed, the harvested RNA from the treated and untreated HepG2 cells were subsequently purified using RNeasy protocol by Qiagen©. All RNA sample concentrations and purities was accessed using UV spectrometry. A 5 ng/µl stock solution was made for all treated and untreated samples and then subsequently stored at 80°C.

RT-PCR Amplification:

The samples were amplified using QIAGEN OneStep RT-PCR kit using 5ng/µl RNA stock. Primer pairs spanning exons 10 through 16 of the primary transcript were used. The thermal cycler steps were performed as at 50°C for 30 minutes, 95°C for 15 minutes. For the 40 cycles it was set for 95°C for 30 minutes, 58°C for 30 minutes, 72°C for 2 minutes, and held at 4°C.

RT-PCR products were visualized on a 1% agarose electrophoresis gel. Bands that were at the expected size or any secondary bands were gel purified using Qiaquick PCR purification kit and sequenced by Genewiz. Identification of splice variants were determined using ApE alignment tool and NCBI BLAST.

Results:

RT-PCR products generated from HepG2 cells treated with compound #135 produced two alternatively spliced variants visualized on an agarose electrophoresis gel (Fig 2).

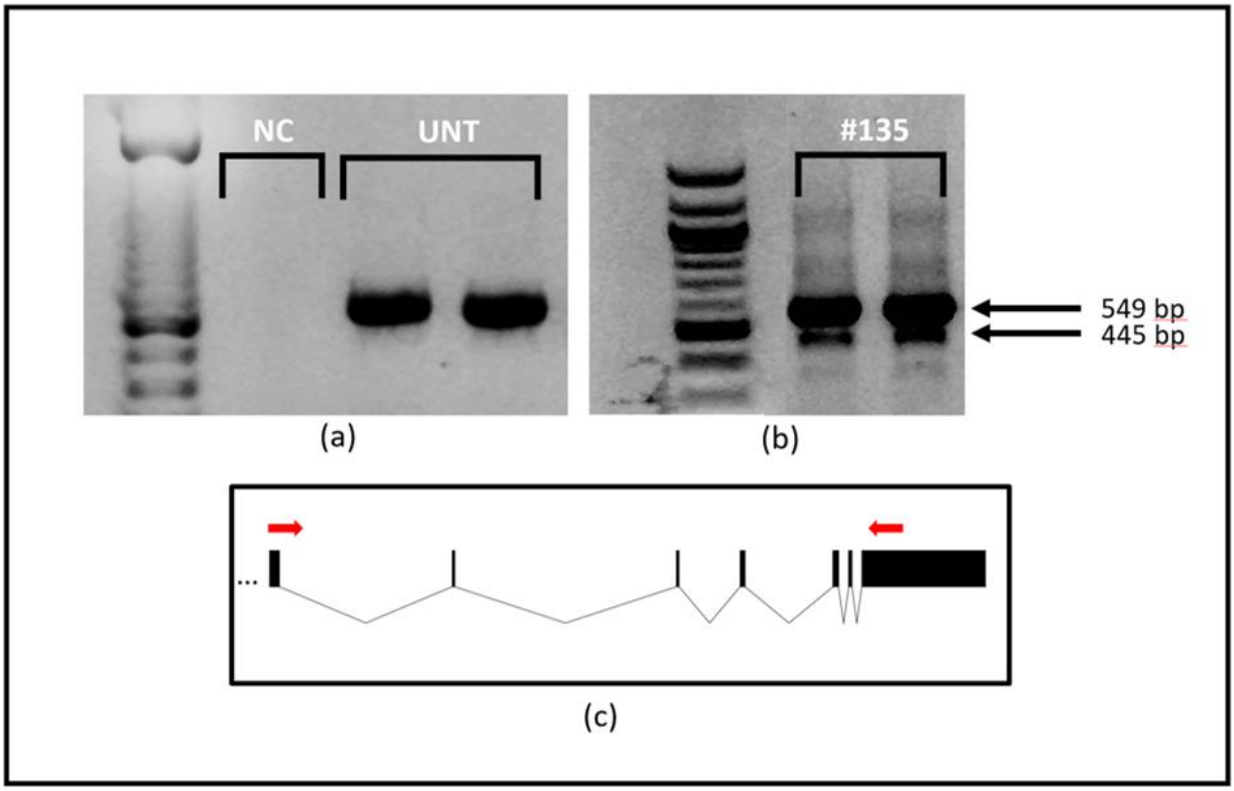

Figure 2. Figure 2- Agarose gel electrophoresis image of RT-PCR products from cells that were untreated (a) and treated with compound #135 (b). RT-PCR products were amplified using primers that spanned exon 10 and exon 16 (c).

Genewiz validated that the two bands were distinctly different RT-PCR products. NCBI Blast determined that the top band with the expected size of 449, included exons 11-16 (Fig 2). However, the lower band RT-PCR product was 445 bp and had low homology when analyzed with NCBI BLAST. ApE alignment tool was used to compare sequences between the suspected

variant and the MAO-A gene sequence. These results indicated that the variant contained all the exons as the expected product, except for exon 14 (Fig 3).

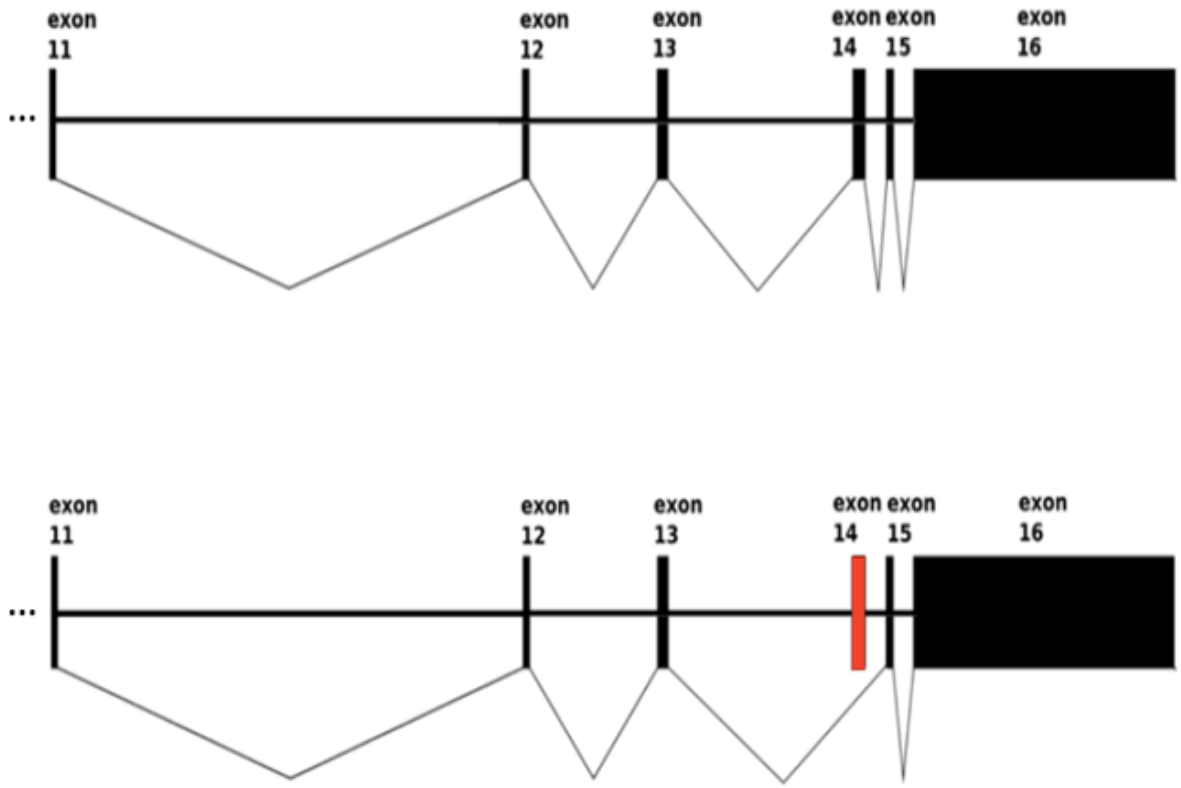

Figure 3: Schematic of normal and alternative spliced variants isolated from cells treated with compound #135.

ExPASY translate tool was used to determine the open reading frame of the spliced variant. Exon 14 exclusion generated a frameshift mutation that resulted in a premature stop codon in exon 15. NCBI was used to ascertain which protein domains were truncated. The variant encodes for a truncated protein in the transmembrane domain (Fig 4).

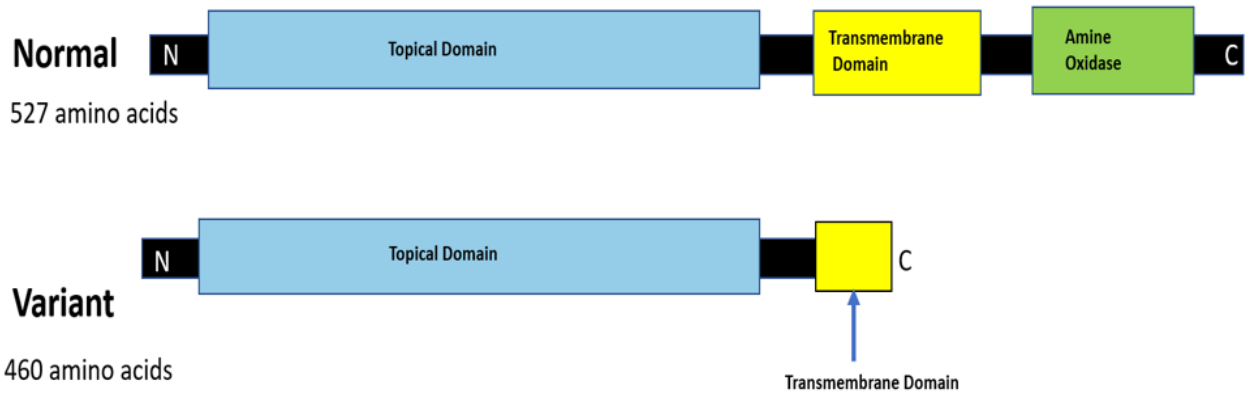

Figure 4: The protein domains for both the normal splice transcript that included all sixteen exons, and the variant transcript of MAO-A that excluded exon 14

Discussion:

Treating HepG2 cells with compound #135 induced alternative splicing in the MAO-A gene transcript. The alternatively spliced variant excluded exon 14 which resulted in a frameshift mutation that coded for a truncated protein. The normal MAO-A transcript encodes a protein that is approximately 527 amino acids long. However, the spliced variant encodes a truncated protein that consists of 460 amino acids. This splice variant has not been recorded on the NCBI database. The truncated protein generated from the splice variant may be less functional. This suggests that the regulation of alternative splicing in the MAO-A gene transcript can be used to regulate MAO-A protein activity. Those with MAO-A associated disorders, such as major depression disorder, can use compounds that induce alternative splicing of MAO-A transcripts that encode less functional protein products.

References:

1. "Mental Disorders: New Insights for the Healthcare Professional: 2013 Edition." Google Books. Ed. Ashton Acton. ScholarlyEditions, 2013. Web. 09 May 2017.

2. European Bioinformatics InstituteProtein Information ResourceSIB Swiss Institute of Bioinformatics. "European Bioinformatics Institute." UniProt. N.p., n.d. Web. 16 May 2017.

3. Sjöberg, R. L., F. Ducci, C. S. Barr, T. K. Newman, L. Dell'osso, M. Virkkunen, and D. Goldman. "A Non-additive Interaction of a Functional MAO-A VNTR and Testosterone Predicts Antisocial Behavior." Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology. U.S. National Library of Medicine, Jan. 2008. Web. 16 May 2017.

4. Fan, M., B. Liu, T. Jiang, X. Jiang, H. Zhao, and J. Zhang. "Meta-analysis of the Association between the Monoamine Oxidase-A Gene and Mood Disorders." Psychiatric Genetics. U.S. National Library of Medicine, Feb. 2010. Web. 16 May 2017.

5. Berlin, I., and R. M. Anthenelli. "Monoamine Oxidases and Tobacco Smoking." The International Journal of Neuropsychopharmacology. U.S. National Library of Medicine, Mar. 2001. Web. 16 May 2017.

6. "Mental Disorders: New Insights for the Healthcare Professional: 2013 Edition." Google Books. Ed. Ashton Acton. ScholarlyEditions, 2013. Web. 09 May 2017.

7. Knott, Dr Laurence. "MAOI Antidepressants. Health and Depression Information." . Health and Depression Information | Patient. Patient.info, n.d. Web. 16 May 2017.

8. Shih, J. C., K. Chen, and M. J. Ridd. "MONOAMINE OXIDASE: From Genes to Behavior." Annual Review of Neuroscience. U.S. National Library of Medicine, 1999. Web. 16 May 2017.

9. "Mental Disorders: New Insights for the Healthcare Professional: 2013 Edition." *Google Books*. N.p., n.d. Web. 16 May 2017.

10. Shih, J. C., K. Chen, and M. J. Ridd. "Role of MAO A and B in Neurotransmitter Metabolism and Behavior." *Polish Journal of Pharmacology.* U.S. National Library of Medicine, n.d. Web. 16 May 2017.