POTENTIAL TREATMENT FOR OBSESSIVE COMPULSIVE DISORDER BY EXPLOITING THE ALTERNATIVE SPLICING OF A GLUTAMATE TRANSPORTER

Saathvika Rajamani
Techniques in Cell and Molecular Biology
Department of Biological Sciences, Fordham University, Bronx, NY

ABSTRACT

Obsessive Compulsive Disorder (OCD) is a chronic anxiety disorder that causes the affected individuals to have recurring thoughts and subsequent repetitive behavior. Obsessions could lead to new compulsions and vice versa. OCD affected individuals have high levels of glutamate neurotransmitter in their cerebrospinal fluids. Genome scan on diseased individuals led to the identification of several polymorphisms in a region in Chromosome 9. The region was later found to correspond to that of gene SLC1A1. This gene codes for a glutamate transporter that transports glutamate into the cells, primarily the neurons. Alternative splicing of this gene having 12 exons results in the production of two unique splice variants that missed either exon 2 or exon 11. These alternative transcripts negatively regulate the primary transcript regulated glutamate transport. Upon treatment with several compounds, the splicing was shown to vary, proving that splicing can be modified based on treatments. Treatment with compound 135 especially showed an increase in splice variant production that missed exon 2. This compound might not be advisable for OCD-affected individuals.

INTRODUCTION

Obsessive Compulsive Disorder (OCD) is a chronic anxiety disorder. It was once thought to be a rare condition, but not so anymore. In fact, the number of OCD affected individuals outranks those affected by other severe mental illnesses such as Schizophrenia and Autism. Research in the previous decades showed a strong influence of genetics on this disorder. This led to twin studies and family based studies of OCD affected individuals that identified polymorphisms in a small region in chromosome 9. This region was further narrowed down to the 24^{th} band in the short arm of this chromosome that coded for a gene, SLC1A1. Solute Carrier 1 Family 1 belongs to a family of solute carriers that are a group of membrane transport proteins. This gene encodes a protein called Excitatory Amino Acid Carrier 1 or Transporter 3 (EAAC1/ EAAT3) which is a glutamate transporter. This glutamate transporter carries glutamate across the plasma membrane of the cell. It is fundamentally an antiporter that imports one molecule of glutamate, a hydrogen ion and three sodium ions while it exports one potassium ion.

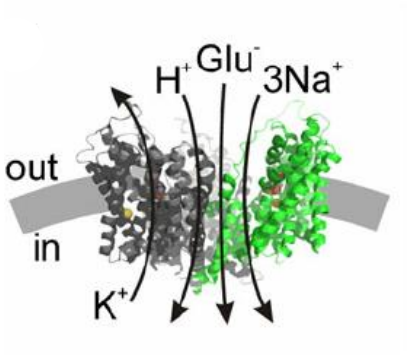

Fig. 1: Excitatory Amino Acid Transporter I encoded by SLC1A1.

The SLC1A1 gene is expressed predominantly in the brain and the kidney. There are many disorders that arise due to the mutations in this gene which include Schizophrenia and Autism. The primary transcript has 12 exons and 11 introns. Three alternative transcripts have been identified

for this gene using bioinformatics tools. They have two promoters, out of which the latter is an internal promoter. Splice variant I has a unique exon upstream of exon 5 and is regulated by promoter 2. The splice variants under the control of promoter 1, II and III, have exon 2 or 11 missing. It is interesting to note that both the exons 2 and 11 code for a transmembrane helix in the amino terminal and the carboxy terminal of the protein respectively.

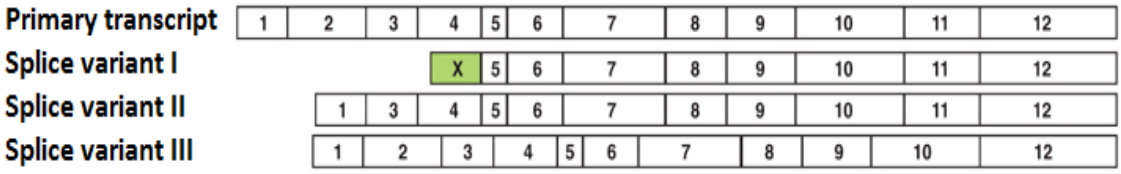

Fig. 2: The splice variants of SLC1A1 gene.

Glutamate is a primary neurotransmitter. High levels of glutamate can induce neurotoxicity and have been linked to various neurodegenerative disorders. The primary transcript encodes for EAAC1 which mediates efficient glutamate transport. This functional glutamate transporter is a trimer or has three subunits. The splice variants II and III encode for proteins that interfere with the formation of the functional EAAC transporter. This leads to a decrease in glutamate transport and an overall increase of glutamate, which further leads to neurotoxicity.

Glutamate levels were found to be comparatively higher in patients suffering from OCD when compared to unaffected individuals upon analyzing the cerebrospinal fluids of both. This indicates improper glutamate transport which could be due to lower production of functional transporters. Thus, lowering the production of these splice variants and/ or increasing the primary transcript production could lead to the formation of functional transporters which would maintain the glutamate levels at equilibrium. The purpose of this project was to identify compounds that upon treating with cells would alter the splicing and hence be a potential cure for OCD.

METHODS

- Human Embryonic Kidney 293 cells or HEK-293 cells were treated with various compounds.
- RNA was isolated from the cells after 24 hours of treatment using the Qiagen RNeasy plus mini kit (QIAGEN, Germany) and purified.
- Primer pairs were designed to span all the exons in the primary transcript. The primer pairs with primers in exon 1 and 8 was chosen in order to focus identification of the splice variant that missed exon 2.
- Reverse Transcriptase PCR was done with these primers on RNA isolated from both untreated and treated samples.
- RT-PCR products were visualized with a UV transilluminator after gel electrophoresis with 1% agarose gel.
- The amplified products were purified using QIAQuick Gel Extraction Kit (QIAGEN, Germany) and sent for sequencing (GENEWIZ, NJ, USA).
- The sequences were analyzed using NCBI-BLAST for confirmation.

RESULTS

RT-PCR was performed on the extracted RNA samples, both treated and untreated, with primers in exons 1 and 8. The treatments that gave interesting results were compounds 135, 54 and 32.

The bands of sizes 846 bp and 777 bp were observed in all the RT-PCR reactions with treated and untreated samples.

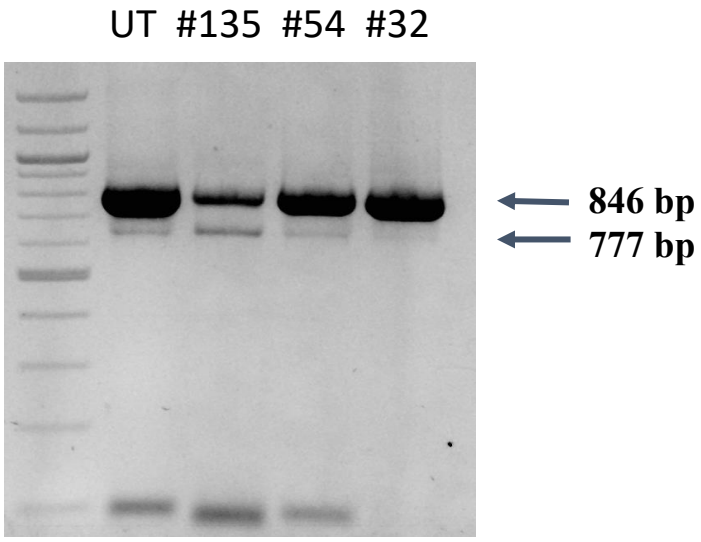

Fig. 3: Agarose gel electrophoresis image of RT-PCR products of treated and untreated RNA samples with SLC1A1 primer pairs for exons 1-8

The RNA sample treated with compound 135 showed the two distinct RT-PCR products upon visualizing in the gel after electrophoresis. The ratio of the two bands was distinctly different, with the primary transcript expressed less and the splice variant expressed more when compared with the other samples.

The other treatments also produced the alternative transcript but it was not biologically significant at such low levels of expression.

Upon gel purification and sequencing, the 846bp transcript was identified as the primary transcript that did not miss an exon. The 777bp was gel purified, sequenced and identified as the splice variant II which was missing exon 2.

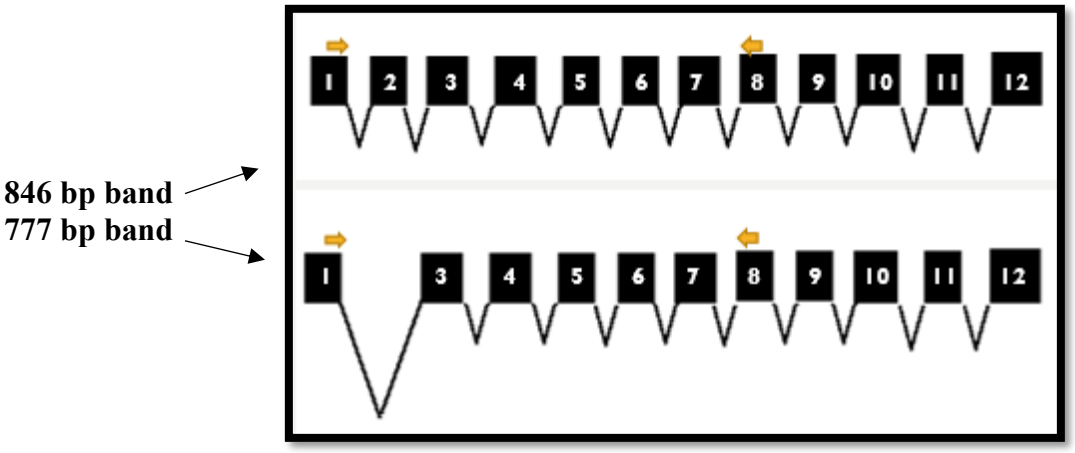

Fig. 4: Diagram of the full-length transcript and the splice variant that is missing exon 2. It also shows the locations of the primers.

DISCUSSION

In this project, the treatment of the HEK 293 cells with certain compounds induced alternative splicing of the transcript of SLC1A1. When the RNA from the cells treated with compound 135 were extracted and amplified, two distinct RT-PCR products were observed. Upon sequencing the products were identified as the primary transcript that did not lack an exon, and the splice variant which lacked exon 2. This splice variant codes for a protein that blocks the formation of the functional glutamate transporter, whereas the primary transcript codes for the functional glutamate transporter itself.

Compound 135 induced higher expression of the splice variant. This would lead to lower formation of functional glutamate transporters and result in less efficient glutamate transport into the cells. This compound should thus be avoided in the diets of the affected individuals.

Overall, this project has shown that compounds have the ability to alternatively splice the SLC1A1 transcript. More compounds would be used in the treatments and protein based assays would also be carried out in the future for further analysis.

REFERENCES

1. Arnold, Paul Daniel, et al. "Glutamate transporter gene SLC1A1 associated with obsessive-compulsive disorder." Archives of general psychiatry 63.7 (2006): 769-776.
2. Dickel, Diane E., et al. "Association testing of the positional and functional candidate gene SLC1A1/EAAC1 in early-onset obsessive-compulsive disorder." Archives of general psychiatry 63.7 (2006): 778-785.
3. Shugart, Y. Y., et al. "A family-based association study of the glutamate transporter gene SLC1A1 in obsessive–compulsive disorder in 378 families." American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 150.6 (2009): 886-892.
4. Grewer, C., Gameiro, A., & Rauen, T. (2014). SLC1 Glutamate Transporters. Pflugers Archiv : European Journal of Physiology, 466(1), 3–24. http://doi.org/10.1007/s00424-013-1397-7