Motor Functions in ALS

Life Sciences Research Program

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Research Question

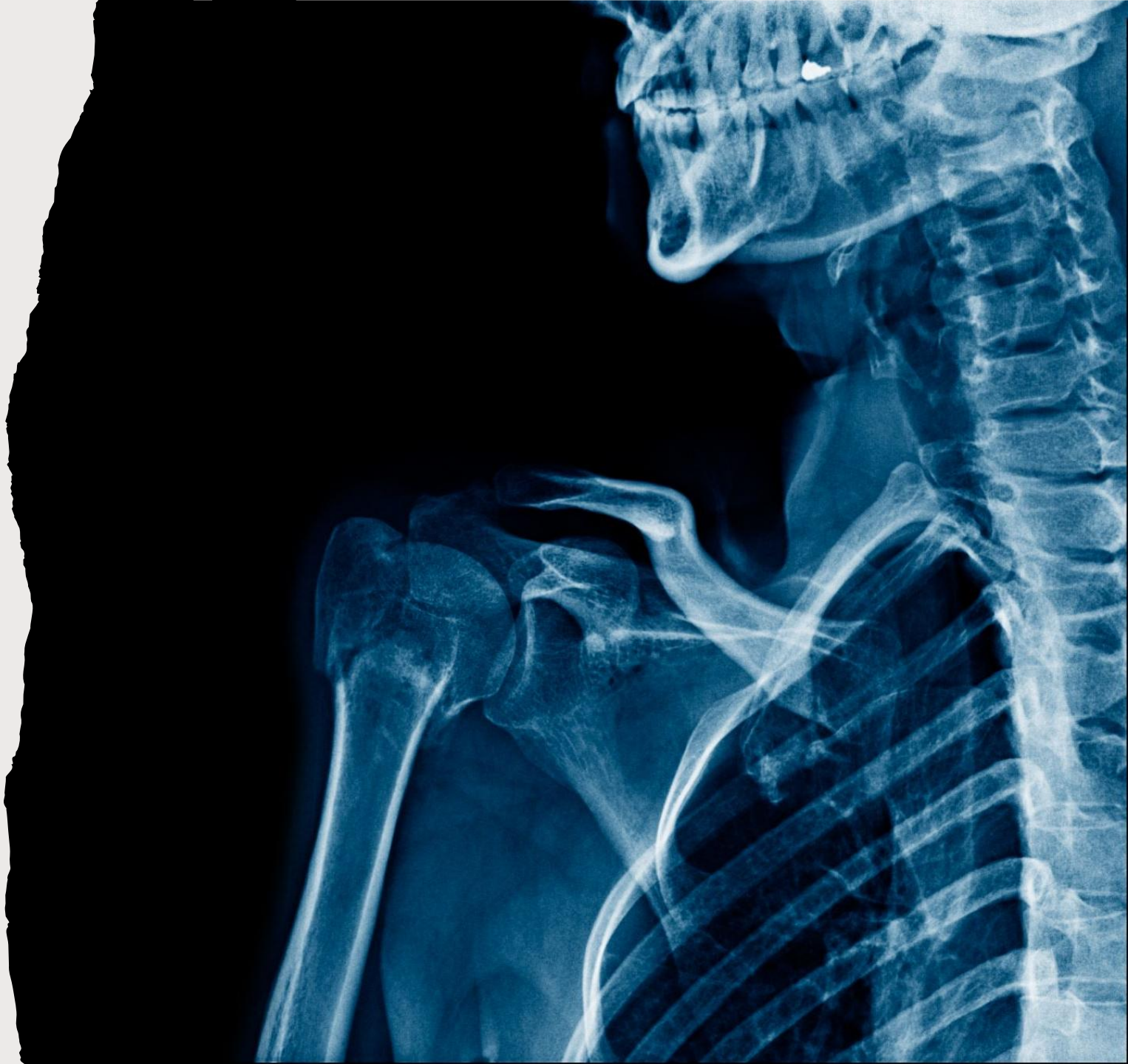
How can neuro-engineering advancements be applied to epigenetics to enhance EAAT2 expression and reduce glutamate expression for those with ALS?

Background Information: ALS

ALS (Lou Gehrig's Disease): Progressive and Fatal neurodegenerative disease

Symptoms:

- Muscle Weakness
- Muscle Spasms
- Fatigue
- Difficulty speaking

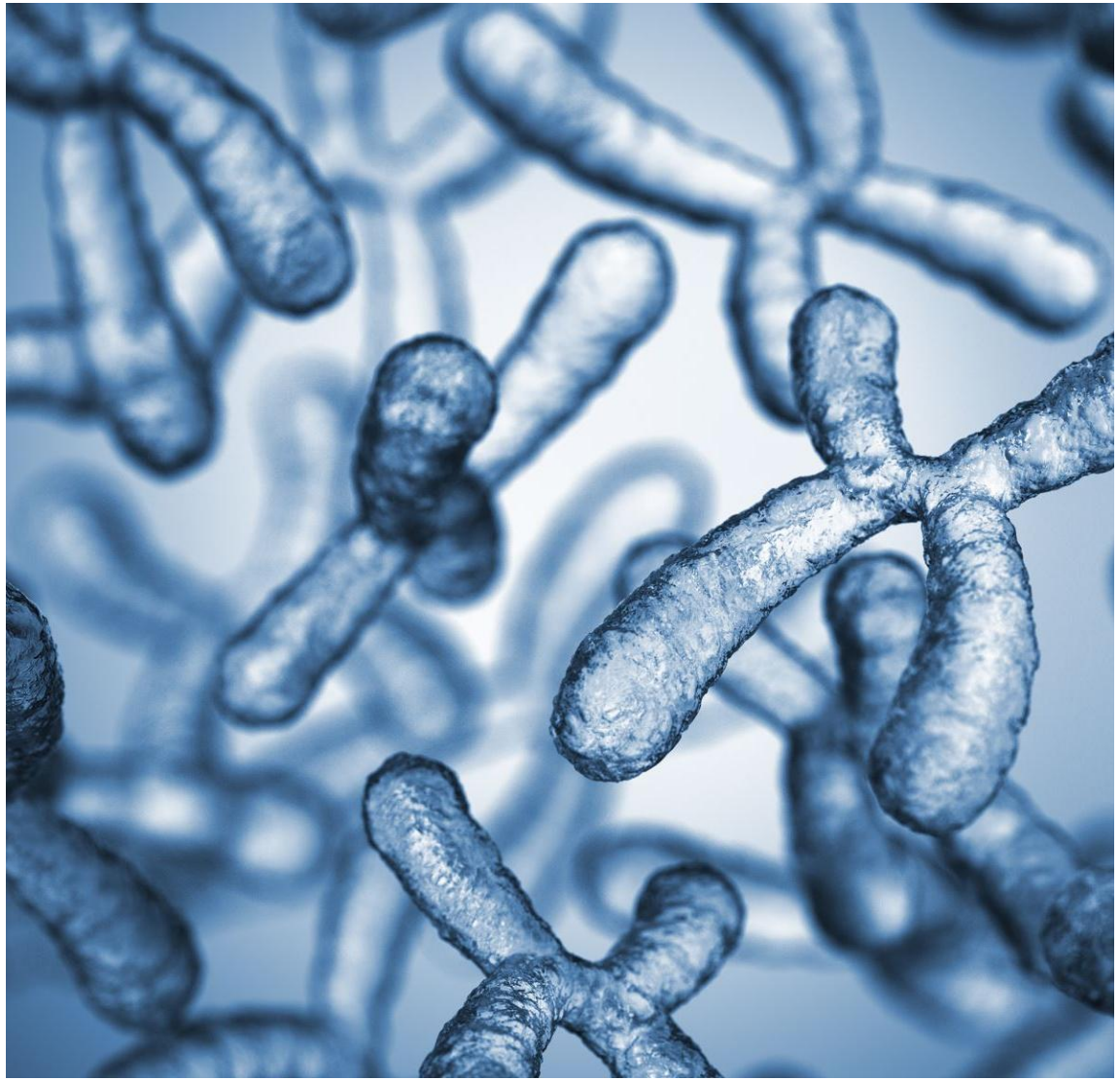


ALS: Neuro-analysis

- Responsible Receptor: **Glutamate Receptors**
- **Key Regions:** Motor Cortex

  Cerebellum

  EAAT2 Gene
- **Clinical Implications:** Overactivation of glutamate receptors can lead to excitotoxicity, contributing to neurodegenerative disorders like ALS



ALS: Neuro-analysis

Astrocytes express the EAAT2 gene which acts as a glutamate transporter – it removes glutamate from the synaptic cleft, preventing it from over-stimulating neurons.

Normal Synaptic Transmission: Astrocytes effecitvely regulate glutamate

Exitotoxic Synaptic Transmission: Impaired EAAT2 gene leads to glutamate accumulation- which leads to neuronal damage that contributes to ALS (Rosenblum, 2017)

Key Question: How can changes within EAAT2 expression affect individuals with ALS?

Unmet Need: Effects of Excitotoxicity of Glutamate

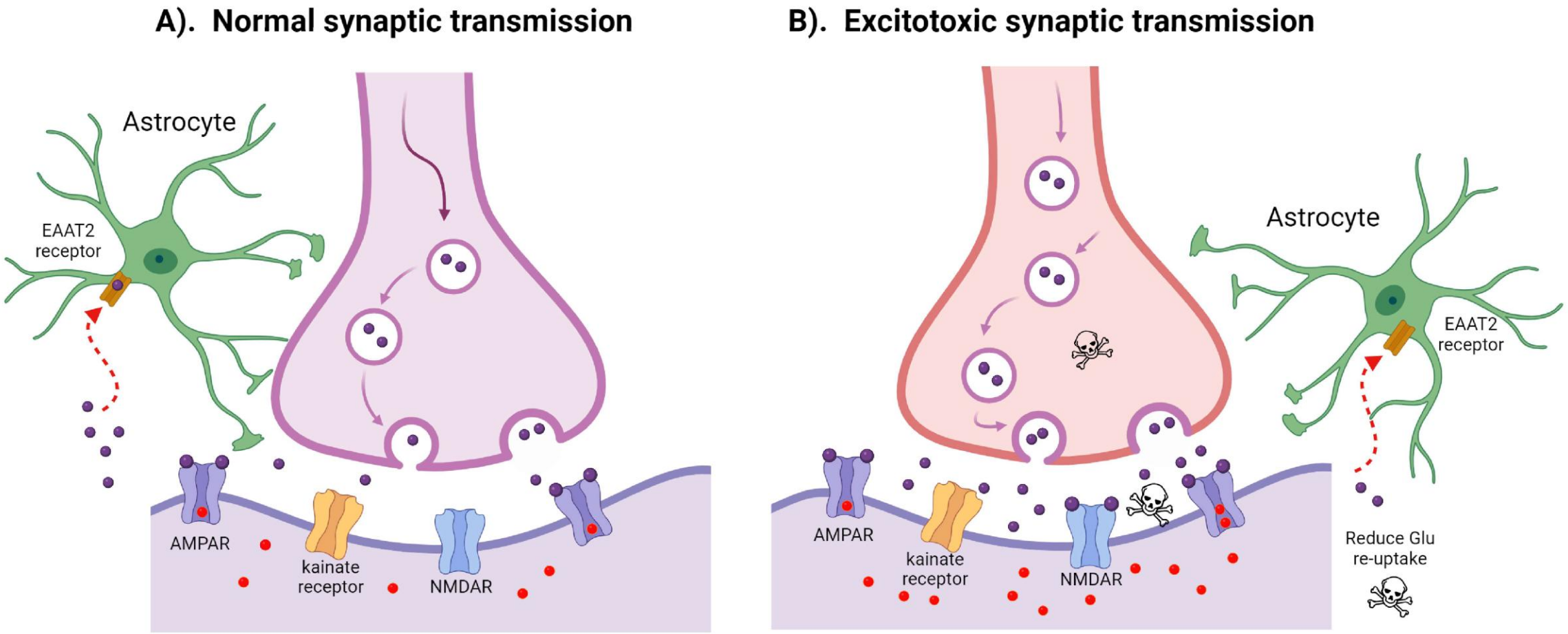


Figure 1: Arnold, F. J., Putka, A. F., Raychaudhuri, U., Hsu, S., Bedlack, R. S., Bennett, C. L., & La Spada, A. R. (2024). Revisiting Glutamate Excitotoxicity in Amyotrophic Lateral Sclerosis and Age-Related Neurodegeneration. *International Journal of Molecular Sciences*, *25*(11), 5587. https://doi.org/10.3390/ijms25115587

Hypothesis

If CRISPRa-based gene methods are used, such as fusing dCas9 with epigenetic modifiers, then it will enhance EAAT2 expression in astrocytes, and extracellular glutamate levels in ALS-affected neural tissue will decrease, thereby reducing excitotoxicity and slowing neuron degeneration.

Methodology: Review-based methodology

CRISPR-a: A Background

Overview: A gene editing tool that allows scientists to modify DNA sequences. It cuts DNA sequences at specific places and adds or removes nucleotides.

Popular Form: CRISPR–Cas9 system is a short RNA molecule that uses guide RNA to target a specific DNA sequence and cleave the DNA at specific locations.

Mutant Form: Dead Cas9 (dCas9)

- Lacks nuclease
- Inactive for DNA Cleavage
- Retains Ability to Bind to DNA
- Regulates Gene Expression by recruiting proteins to target site

Significance: The dCas9 can also be fused with an epigenetic modifier to directly manipulate the epigenetic states at the enhancer region, thereby activating the targeted genes (Chen, 2017)

Epigenetic Regulation

Background: Epigenetics refers to changes in gene expression without changes in the DNA sequences. It includes two types: **Histone Acetylation and DNA Demethylation**

Histone Acetylation

Histone acetyltransferases modify histone proteins by adding acetyl groups to histone tails, which can enhance transcription. This project focuses on the histone acetyltransferase p300 domain.

DNA Demethylation

The addition of methyl groups can inactivate a gene, and is catalyzed by enzymes called DNA methyltransferases. Conversely, the removal of groups can activate transcription factors. This DNA demethylation involves the TET Family which serves as a key intermediate in the demethylation pathway (An, 2017).

Activation Method 1: *histone acetyltransferase p300*

Solution: dCas9 fused to histone acetyltransferase p300 (dCas9-p300) activation domain (Figure 2)

Explanation: The dCas9-p300 system can be crucial for enhancing EAAT2 gene expression. The p300 HAT domain is responsible for transferring acetyl groups to lysine residues in histone tails (Zhang, 2014). The dCas9 protein can be fused to the p300 system and then the dCas9-p300 system can target the EAAT2 gene. This process will recruit the p300 domain and lead to acetylation, which will ultimately lead to activation (Hilton, 2015).

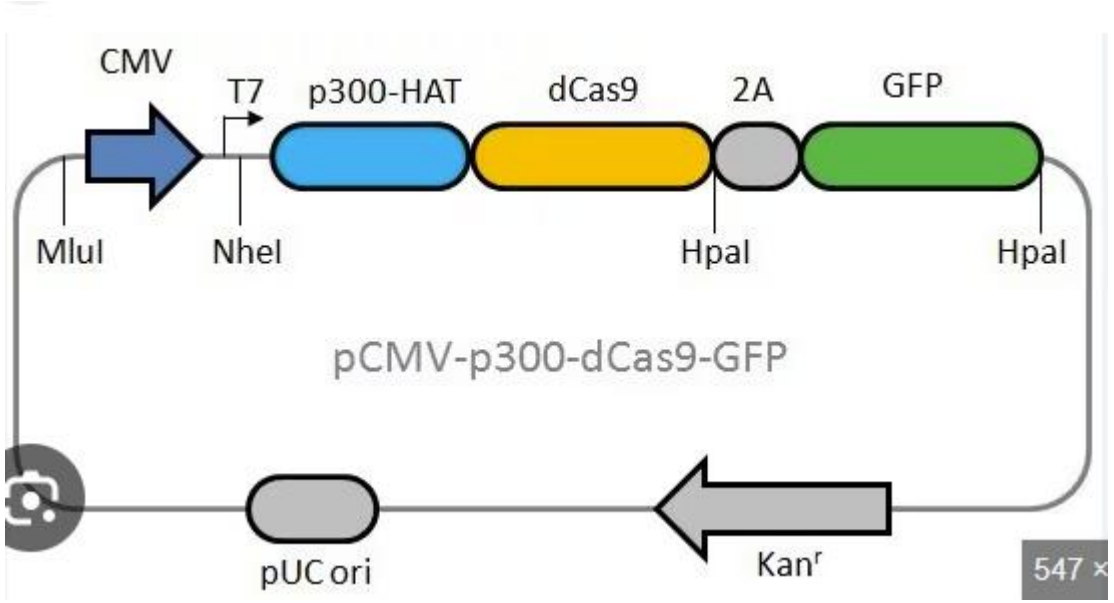


Figure 2: Hilton IB, D'Ippolito AM, Vockley CM, Thakore PI, Crawford GE, Reddy TE, Gersbach CA. 2015. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat Biotechnol.* 33(5):510-517. https://doi.org/10.1038/nbt.3199

Activation Method 2: *DNA Demethylase Through TET*

Background: A study of a DNA methylation profile revealed a link between EAAT2 expression and the methylation of the ***CpG island*** located within the EAAT2 promoter (Zschocke, 2007). EAAT2 genes that express normal expression had a lack of methylation within these promoter genes, whereas EAAT2 genes that express a lack of expression had hypermethylation within the CpG island.

Solution: The dCas9 fused with the TET Catalytic domain can be used to prevent hypermethylation of the CpG island within the EAAT2 Gene.

Explanation: The TET Catalytic domain oxidizes 5mC to 5-hydroxymethylcytosine, which is a process essential to DNA demethylation (Tan, 2012). Fusing the TET domain to dCas9 can allow targeted demethylation of the CpG islands within the EAAT2 Gene which can result in transcription and increased expression.

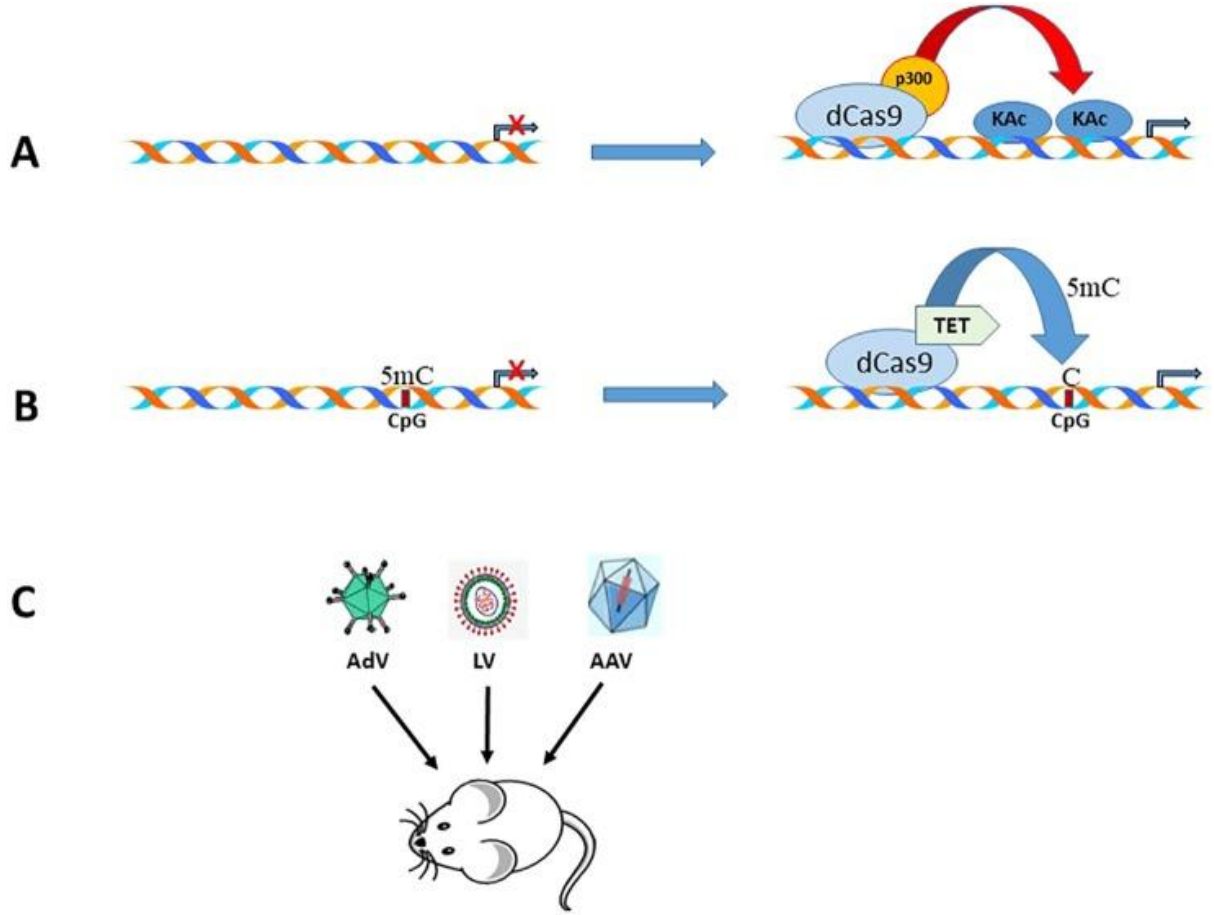


Figure 3: In Mohammad Alams study of CRISPR funtion in epigenetics, there is a successful demonstration of CRISPR to activate acetylation and demethylation is noted amongst rodents. A demonstrates the process of the dCas9-p300 system for acetylation, and B demonstrates the use of CRISPR fused with TET to lead to DNA demetyhlation.

Alam MA, Datta PK. Epigenetic Regulation of Excitatory Amino Acid Transporter 2 in Neurological Disorders. Front Pharmacol. 2019 Dec 13;10:1510. doi:10.3389/fphar.2019.01510. PMID: 31920679; PMCID: PMC6927272.

Limitations

Technical Limitations

Cost and Scalability

Ethical Considerations

Conclusion

DCAS9 FUSED TO (A) HISTONE ACETYLTRANSFERASE P300 (DCAS9-P300) ACTIVATION DOMAIN (HILTON ET AL., 2015)

DNA DEMETHYLASE CATALYTIC DOMAIN FROM THE TET FAMILY (XU ET AL., 2016).

END RESULT: ACTIVATION OF THE EAAT2 GENE WILL LEAD TO REDUCED GLUTAMATE ACCUMULATION, WHICH CAN PREVENT ALS.

References

- Alam MA, Datta PK. (2019). Epigenetic Regulation of Excitatory Amino Acid Transporter 2 in Neurological Disorders. *Front Pharmacol, 10*, 1510. https://doi.org/10.3389/fphar.2019.01510. PMID: 31920679; PMCID: PMC6927272.
- An J, Rao A, Ko M. (2017). TET family dioxygenases and DNA demethylation in stem cells and cancers. *Exp Mol Med, 49*(4), e323. https://doi.org/10.1038/emm.2017.5. PMID: 28450733; PMCID: PMC6130217.
- Arnold, F. J., Putka, A. F., Raychaudhuri, U., Hsu, S., Bedlack, R. S., Bennett, C. L., & La Spada, A. R. (2024). Revisiting Glutamate Excitotoxicity in Amyotrophic Lateral Sclerosis and Age-Related Neurodegeneration. *International Journal of Molecular Sciences*, *25*(11), 5587. https://doi.org/10.3390/ijms25115587
- Chen M, Qi LS. (2017). Repurposing CRISPR System for Transcriptional Activation. *Adv Exp Med Biol, 983*, 147–157. https://doi.org/10.1007/978-981-10-4310-9_10. PMID: 28639197; PMCID: PMC10211423.
- Gostimskaya I. (2022). CRISPR-Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. *Biochemistry (Mosc), 87*(8), 777–788. https://doi.org/10.1134/S0006297922080090. PMID: 36171658; PMCID: PMC9377665.
- Hilton IB, D'Ippolito AM, Vockley CM, Thakore PI, Crawford GE, Reddy TE, Gersbach CA. (2015). Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat Biotechnol, 33*(5), 510–517. https://doi.org/10.1038/nbt.3199. PMID: 25849900; PMCID: PMC4430400.
- Johns Hopkins Medicine. (n.d.). *Amyotrophic lateral sclerosis (ALS)*. https://www.hopkinsmedicine.org/health/conditions-and-diseases/amyotrophic-lateral-sclerosis-als
- Redman M, King A, Watson C, King D. (2016). What is CRISPR/Cas9? *Arch Dis Child Educ Pract Ed, 101*(4), 213–215. https://doi.org/10.1136/archdischild-2016-310459. PMID: 27059283; PMCID: PMC4975809.
- Rosenblum LT, Trotti D. (2017). EAAT2 and the Molecular Signature of Amyotrophic Lateral Sclerosis. *Adv Neurobiol, 16*, 117–136. https://doi.org/10.1007/978-3-319-55769-4_6. PMID: 28828608; PMCID: PMC6668619.
- Tan L, Shi YG. (2012). Tet family proteins and 5-hydroxymethylcytosine in development and disease. *Development, 139*(11), 1895–1902. https://doi.org/10.1242/dev.070771. PMID: 22569552; PMCID: PMC3347683.
- Zhang X, Ouyang S, Kong X, Liang Z, Lu J, Zhu K, Zhao D, Zheng M, Jiang H, Liu X, Marmorstein R, Luo C. (2014). Catalytic mechanism of histone acetyltransferase p300: from the proton transfer to acetylation reaction. *J Phys Chem B, 118*(8), 2009–2019. https://doi.org/10.1021/jp409778e. PMID: 24521098; PMCID: PMC4037238.
- Zschocke J, Allritz C, Engele J, Rein T. (2007). DNA methylation dependent silencing of the human glutamate transporter EAAT2 gene in glial cells. *Glia, 55*(7), 663–674. https://doi.org/10.1002/glia.20497. PMID: 17311293.