**Research Question & Aim**

It has been shown that VitD3 has a proven relationship with delaying symptoms of the onset of psychosis-related symptoms (1). With this, it has also been suggested that a certain subgroup of AD patients bear genetic risk alleles which allow for vitD3 to induce benefits to their progression of Alzheimer’s disease (AD) (2).

The aim of this study is to investigate the effect of Vitamin D3 on the genetic expression and variation in AD patients. We intend to identify underlying changes within the pathway and network of Alzheimer’s disease progression affected by the presence of vitamin D. As well, we plan to identify which genetic risk alleles AD patients must bear to benefit from VitD3 as a psychosis-related treatment.

**Research Methods to be Used:**

1. Firstly, the gene expression datasets need to be accessed, this will probably be done with the **Gene Expression Omnibus database** (http://www.ncbi.nlm. nih.gov/geo/). Raw gene expression can be obtained from GEO.
2. Once the genetic data is obtained, the effect of VitD3 in AD patients will be observed, processed and statistically analysed using **R Studio/Bioconductor**. Transcriptomic data is often received in the form of comparative data, such as normal (controlled) expression values vs altered (perturbed) expression data.
   1. The potential implementation of PATHChange, a package in R specially developed to identify differently expressed patterns in transcriptomic data, could be useful. It uses three different statistical tests: Bootstrap, Fisher Exact and Wilcoxon signed rank tests, with these it evaluates alterations that are considered significant and hopefully reduces probability of false discoveries.
3. This processed dataset will then be transferred to PathVisio. Pathway analysis with **Pathvisio** will be done to identify significant alterations and changes in biological processes due to varying genetic expression related to VitD3 metabolism and AD progression.
4. A network illustrating the relevant molecular alterations and genetic variation taking place will be created and analysed, this will be done with the open source tool for network analysis, **Cytoscape**.
   1. Within the open source analysis tool, the **ClueGO app** could possibly be used to analyse and group sets of genes to identify correlated groups.
5. Sequence-based analysis will be done to investigate if the genetic response element for vitamin D (VDRE) is present in the promoter regions of genes related to AD progression.
   1. CS - BLAST – Sequence Context Specific BLAST (Search of Nucleotide, Protein, and Genome databases) could be used for this section of methods to compare sequences.
6. Wang L, Ying J, Fan P, Weamer E, DeMichele-Sweet M, Lopez O, et al. Effects of Vitamin D Use on Outcomes of Psychotic Symptoms in Alzheimer Disease Patients. The American Journal of Geriatric Psychiatry. 2019;27.
7. Dursun E, Gezen-Ak D. Vitamin D basis of Alzheimer’s disease: from genetics to biomarkers. Hormones. 2019;18(1):7-15.