RESEARCH PROJECT PROPOSAL

SYNAPTIC miRNAs AS POTENTIAL BIOMARKERS FOR ALZHEIMER'S DISEASE

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BACKGROUND

Alzheimer's Disease (AD) is currently conceived as a continuum that can be characterized by performing combined assays, including neuropathological findings and AD-specific biomarkers - CSF (t-Tau, p-Tau, Aβ42) and neuroimaging biomarkers (MRI, FDG-PET, Aß-PET). However, invasiveness, high cost, and insufficient availability of current biomarkers limit their use in routine clinical practice for AD diagnosis, therefore a refocusing towards the identification of blood-based biomarkers has been proposed. [1]

Moreover, due to high stability in biofluids and easy quantification, the identification of altered miRNA expression profiles in AD patients represents a promising approach for developing novel AD-specific blood-based biomarkers.

Within this research proposal, the analysis of synaptic miRNAs is suggested as a potential AD-biomarker, since synaptic impairment has been recently identified as an upstream event in AD pathology, which precedes neuronal loss.

This project aims to propose and experimentally validate a novel plasma miRNA signature that accurately distinguishes between the different stages of AD, and therefore that could serve as a reliable biomarker for the detection of AD during its early stages.

SYNAPTIC miRNAs PROPOSAL

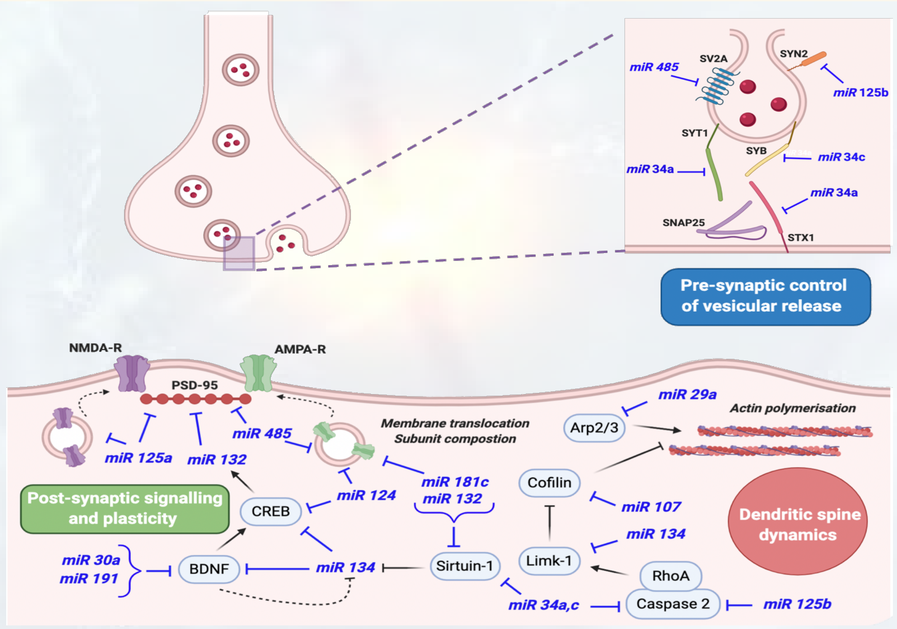

Figure 1: Overview of proposed miRNAs at the synaptic compartment.
Abbreviations: SYN2, Synapsin 2; SNAP25, Synaptosomal-associated protein 25; SYT1, synaptotagmin 1; STX1, syntaxin 1; SV2A, Synapse vesicle 2A; SYB, Synaptobrevin; Arp2/3, Actin-related protein 2/3. [2]

PROJECT DEVELOPMENT

For the project development, a bibliographic research has been conducted on miRNAs involved in normal synaptic function, and its analysis in clinical samples:

- The most used databases were PubMed, and MiRBase.
- Research key words: "AD blood-based biomarkers", "miRNAs in AD", "miRNAs and synaptic function", "miRNA-guided diagnosis".

HYPOTHESIS AND OBJECTIVES

Alterations in the plasma expression profile of proposed miRNAs can be useful as a reliable non-invasive AD-specific biomarker for an early-diagnosis of AD

- To evaluate the diagnostic capacity of proposed miRNAs in plasma as potential AD-biomarkers, and estimate their disease specificity versus other neurodegenerative diseases.
- To determine whether variations in the plasma expression profile of these miRNAs provide an insight into AD's pathological changes in the brain tissue.
- To examine whether these miRNAs present a similar expression pattern in both plasma and CSF.

STUDY DESIGN AND DEVELOPMENT PLAN

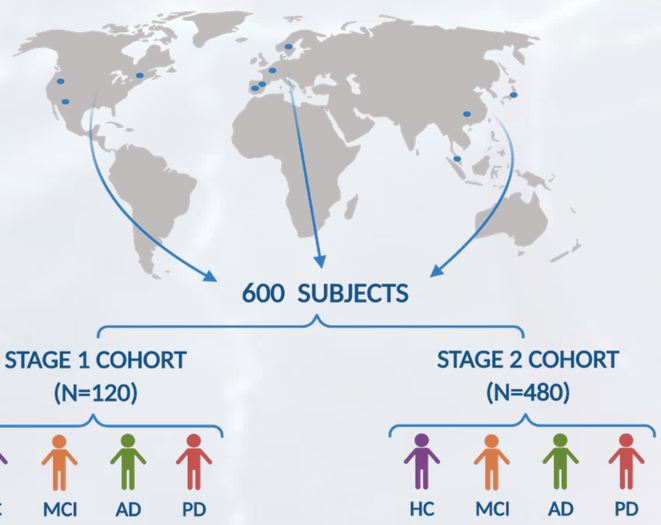

Figure 2: An internationally two-stage cross-sectional study.
600 individuals will be recruited from hospitals in countries with a high incidence of AD, and distributed into the following groups according to the inclusion criteria; healthy controls (HC), mild cognitive impairment due to AD (MCI), Alzheimer's Disease (AD), and Parkinson's Disease (PD).

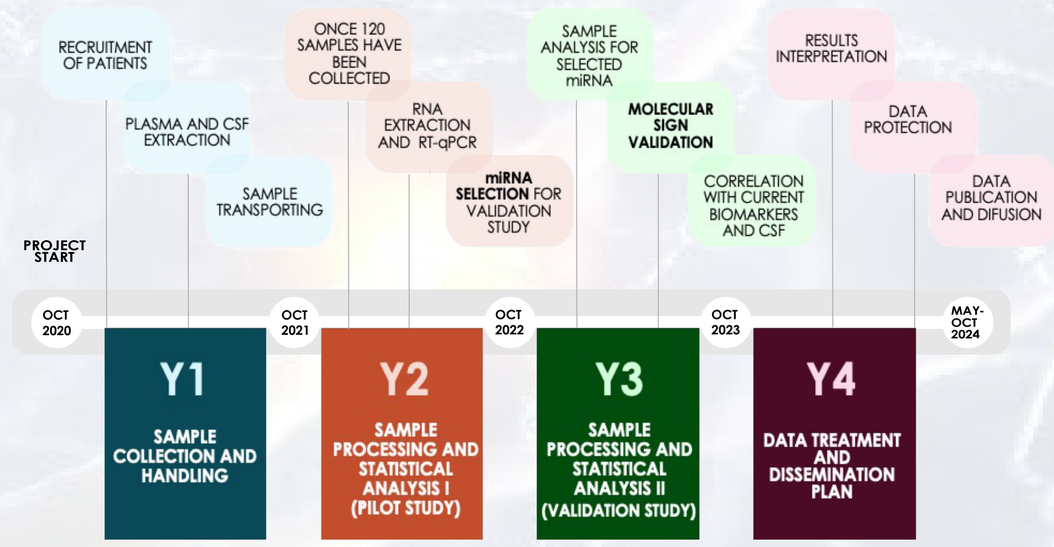

Figure 3: Project timeline.
Scheme representing the project work-flow through a four-year development plan, and outlining the study design into two different stages: stage 1 or pilot study and stage 2 or validation study.

EXPERIMENTAL PROCEDURES AND EXPECTED RESULTS

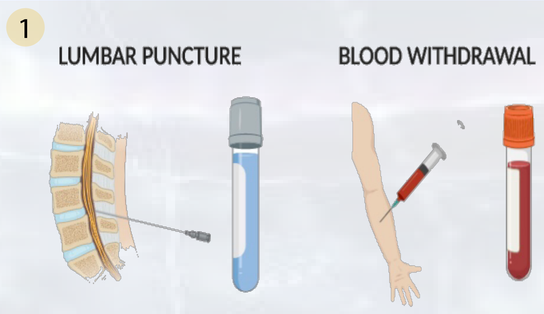

Figure 4: Sample collection and Handling.
Specimens will be collected and processed following the preanalytical standard operating procedures of BIOMARKAPD consortium.

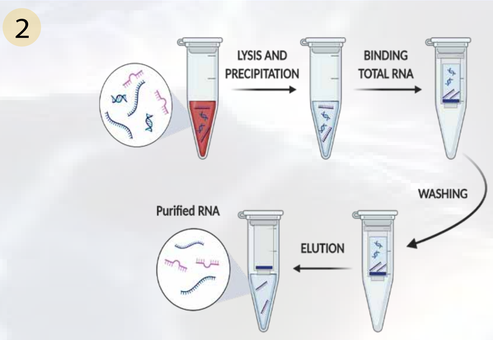

Figure 5: RNA extraction.
It will be performed using miRNeasy Serum/Plasma Advanced Kit (Qiagen). *C.elegans* miRNA cel-miR-39 (Qiagen) will be used as an internal reference for normalization. [3]

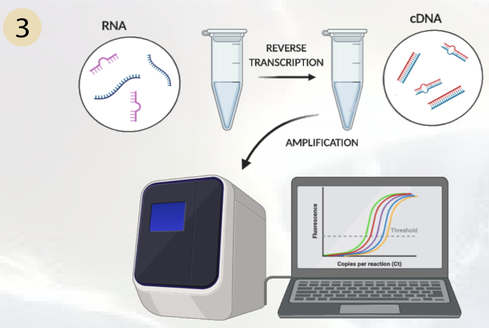

Figure 6: Measurement of miRNAs levels by RT-qPCR assays.
Purified miRNA will be reversed transcribed to cDNA using TaqMan miRNA Reverse Transcription Kit (Applied Biosystems) and amplified by qPCR. [3]

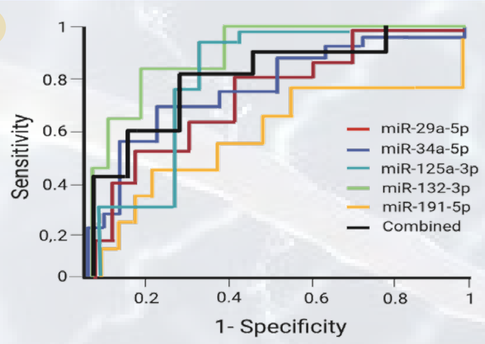

Figure 7: miRNA signature validation.
ROC curve assays will be performed for the evaluation of miRNA diagnostic capacity. This image shows an example of a ROC curve analysis for selected miRNA in MCI.

CONCLUSIONS

Nowadays, to establish an early diagnosis of AD is essential to advance in its therapeutic approach for several reasons:

- To increment the effectiveness of available treatments.
- To allow individuals to establish their health-care programs and decide about important matters before becoming demented.
- To provide time to perform multiple interventions for the early prevention of cognitive decline.

Therefore, this research proposal represents the first step in the development of a consistent, non-invasive biomarker that could assist in the routine clinical practice for AD diagnosis in its early stage.

REFERENCES

1 Nagaraj S, Zoltowska KM, Laskowska-Kaszub K, Wojda U. microRNA diagnostic panel for Alzheimer's disease and epigenetic trade-off between neurodegeneration and cancer. Ageing Res Rev. 2019 Jan 1;49:125–43.

2 Millan MJ. Linking deregulation of non-coding RNA to the core pathophysiology of Alzheimer's disease: An integrative review. Prog Neurobiol. 2017 Sep 1;156:1–68.

3 Gustafson D, Tyryshkin K, Renwick N. microRNA-guided diagnostics in clinical samples. Best Pract Res Clin Endocrinol Metab. 2016 Oct 1;30(5):563–75.