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Monitoring drug Efficacy through Multi-Omics Research initiative in Alzheimer's Disease (MEMORI-AD): A protocol for a multisite exploratory prospective cohort study on the drug response-related clinical, genetic, microbial and metabolomic signatures in Filipino patients with Alzheimer's disease

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ABSTRACT

Introduction Dementia is one of the leading causes of disability among older people aged 60 years and above, with majority eventually being diagnosed with Alzheimer's disease (AD). Pharmacological agents approved for dementia include acetylcholinesterase enzyme (AChE) inhibitors like rivastigmine, donepezil and galantamine and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine, prescribed as monotherapy or in combination with each other, depending on the severity of disease. There is currently no available study demonstrating the clinical response to these drugs for AD in the Filipino population. Hence, this protocol aims to characterise the clinical, genetic, microbial and metabolic factors associated with drug responses to donepezil, rivastigmine and/or memantine for AD in a cohort of Filipinos with late-onset AD.

Methods and analysis This protocol involves a multisite descriptive study that will use two study designs: (1) a descriptive, cross-sectional study to characterise the clinical profile of Filipino dementia patients with AD and (2) an exploratory prospective cohort study to investigate drug response-related genetic, gut microbiome and metabolome signatures of a subset of the recruited AD patients. At least 153 patients with mild or moderate AD aged 65 years old and above will be recruited regardless of their treatment status. A subset of these patients (n=60) who meet inclusion and exclusion criteria will be included further in the exploratory cohort study. These patients will be grouped according to their baseline medications and will be observed for treatment response in 6 months. The cognitive, functional and behavioural domains of patients and levels of functioning will be measured using different assessment tools. Drug responses of Filipino patients will then be investigated employing multi-omics technology to characterise genetic variations via whole exome sequencing, gut microbiome profile via shotgun metagenomic sequencing and metabolome profile via liquid chromatography with mass spectrometry.

Ethics and dissemination The study has received ethical clearance from the Department of Health Single Joint Research Ethics Board (SJREB-2022–15). Results of psychometric scales will be made available to enrolled patients. The study results will be presented at national/

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study will provide the first multisite, comprehensive cohort study to investigate the clinical, genetic, microbial and metabolomic factors associated with pharmacologic response to anti-dementia drugs of Filipinos with late-onset Alzheimer's disease (AD).
⇒ This study will provide crucial evidence on the real-world treatment regimen, leading to the future development of local clinical practice guidelines in managing patients with AD.
⇒ The primary limitation of this study is the need for a healthy control group.

international conferences and published in international peer-reviewed scientific journals, and summaries of the results will be provided to the study funders and institutional review boards of the three tertiary referral hospitals.

Trial registration number Philippine Health Research Registry ID PHRR230220-0054116; ClinicalTrials.gov ID NCT05801380

INTRODUCTION

Dementia is one of the leading causes of disability and dependency among older people, affecting more than 55 million people worldwide.[1] It is characterised by the progressive decline in cognitive domains, including memory, executive function, language, visuospatial abilities, personality and behaviour.[2] The Diagnostic and Statistical Manual of Mental Disorders (DSM-5-TR) criteria defines dementia or major neurocognitive disorder as a significant decline from a previous level of functioning in at least one cognitive domain, interfering with the individual's independence in activities of daily living.[3] Dementia may occur as early-onset (<65 years) or late-onset (≥65 years), with more than 97% of cases being late-onset.[4]

Alzheimer's disease (AD) is the most common type of dementia, contributing to 60–70% of all dementia cases.[1] In the Philippines, data on the prevalence of AD is limited, with only one single-city study published in the literature.[5] There are several clinical criteria for diagnosing AD, such as the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria,[6] DSM-5-TR criteria for AD[3] and the International Working Group 2 criteria.[7] In the Philippines, the NIA-AA criteria,[6] National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria[8] and DSM-5-TR criteria[3] along with imaging studies are often used for the clinical diagnosis of AD.

The clinical manifestations of AD are currently attributed to excessive levels of amyloid beta (Aβ) deposits, tau-containing neurofibrillary tangles and atrophy of the brain,[9] causing mitochondrial dysfunction and disruption of axonal transport, which in turn results in synaptic dysfunction, neurodegeneration and cognitive dysfunction.[10]

Despite the increasing cases of dementia and AD each year, there is still no definite cure for AD. Currently, available drugs can only provide symptomatic therapy and have little effect in preventing or reversing the neurodegenerative process in AD.[11] To date, there are only two classes of drugs commonly used and included in the Philippine National Drug Formulary[12] for AD treatment in the Philippines: acetylcholinesterase enzyme (AChE) inhibitors (ie, donepezil, rivastigmine and galantamine) for mild to severe AD and the N-methyl-D-aspartate (NMDA) receptor antagonist (memantine) for moderate to severe AD.[2 13] Table 1 summarises the recommended AD treatment in the Philippines.

Recently, monoclonal antibodies have received attention as a potential treatment for AD by eliminating toxic Aβ species and thereby preventing neurodegeneration and cognitive decline.[14] However, there has been no success with amyloid-targeting treatment in slowing the course of cognitive impairment in symptomatic AD. Moreover, the correlation between phases of amyloid deposition and decline in cognitive function has been minimal,[15] and no amyloid beta-directed monoclonal antibody is currently available in the Philippines.

Omics study of AD

Multiomics approaches have revolutionised our understanding of AD, including its pathophysiology, disease progression and drug response. Recent research advances in these multiomics, including genomics, transcriptomics, microbiome and metabolomics have pushed for a holistic understanding of AD. Genomic studies, with the use of next-generation sequencing and genome-wide association studies, have expanded the knowledge on genetic risk factors of disease progression, identifying ApoE, SORL1, BIN1, TREM2 and ABCA7 for disease pathology and drug response variability.[16 17] Along with these genomic studies are transcriptomic analyses, which contributed to knowledge of gene expression patterns in AD and drug response.[18 19]

Beyond genetic factors causing the disease, the gut-brain axis has emerged as one of the most important areas of investigation in AD. Recent studies show that the gut microbiota composition is changed not only in AD

Table 1 Pharmacotherapy in Alzheimer's disease[51]

Severity of Alzheimer's disease	Standard recommendation
Mild	Acetylcholinesterase inhibitors (donepezil, rivastigmine)*
Moderate	Acetylcholinesterase inhibitors (donepezil, rivastigmine)* **AND/OR** NMDA antagonist (memantine)
Severe	Acetylcholinesterase inhibitors (donepezil, rivastigmine)* **AND/OR** NMDA antagonist (memantine)

*Galantamine was not included because it is no longer marketed in the Philippines.
NMDA, N-methyl-D-aspartate.

patients but also in correlation with amyloid plaque formation and neuroinflammation.[20 21] Such findings point out microbial metabolites, like short-chain fatty acids and lipopolysaccharides, as possible key players of brain function modulation and drug response.[22] For instance, *Bacteroides* species, which have been identified to be elevated in AD, are implicated in the pathogenesis of AD,[23] while specific probiotics exert their effects in improving symptoms of AD by altering the gut microbiota.[24] Moreover, multiomics approaches now identify how medications for AD interact with the gut microbiota and, therefore, their possible influence on the treatment response.[25] This last area of research has only recently started.

Another fast-growing area in AD research, metabolomics provides insights into the metabolic dysregulation featured in the disease. Nuclear magnetic resonance and mass spectrometry techniques have allowed the identification of important metabolites that might be implicated in the pathogenesis of AD.[26 27] For example, lipid metabolism has been linked to several aspects of AD development, including amyloid production, oxidative stress and myelin degeneration.[28] Concurrently, therapeutic interventions targeting these identified pathways are also in development.[28] Moreover, potential biomarkers for the identification of disease and treatment monitoring have already resulted from these metabolomics studies.[29 30]

Despite these advancements, there remains a lack of studies specifically investigating the multiomics profiles of Filipino AD patients. Genetic diversity and unique environmental exposures, including diet and microbiome composition, may influence drug responses in ways that are not captured by studies in predominantly Western populations. This underscores the need for multiomics studies in diverse populations, especially in the global south, to better understand interindividual variability in AD drug response and to develop more effective therapeutic strategies.

Objectives

The study aims to characterise the clinical, genetic, microbial and metabolomic factors associated with drug response to an AChE inhibitor (donepezil, rivastigmine) and NMDA receptor antagonists (memantine) given for AD in a cohort of Filipinos with late-onset AD. Specific objectives include (a) describing the clinical profile of Filipino patients with AD, (b) identifying drug response-related gene signatures specific to Filipino AD patients, (c) investigating drug response-related gut microbiome signatures of Filipino AD patients, and (d) investigating drug response-related metabolome signatures of Filipino AD patients.

METHODS AND ANALYSIS

Study design

This protocol will use two study designs: (1) a descriptive, cross-sectional study design to characterise the clinical profile of Filipino patients with AD in three tertiary referral hospitals and (2) an exploratory prospective cohort study design to investigate drug response-related genetic, gut microbiome and metabolome signatures of a subset of the recruited AD patients (figure 1) who will be observed for treatment response for 6 months. The descriptive, cross-sectional design will allow for the characterisation of the clinical profile of Filipino AD patients, providing a baseline understanding of the population in terms of cognitive, functional and behavioural features. This information is critical in the context of precision medicine, where such demographic-specific baselines are important. Meanwhile, the exploratory prospective cohort design will be used to monitor drug response longitudinally, capturing the temporal variations in the gut microbiome, and metabolomic signatures that may influence therapeutic outcomes.

Study period and sites

The study started in January 2022 and will be implemented until February 2025 in the outpatient Adult Neurology clinic of three tertiary hospitals within the National Capital Region: the University of the Philippines—Philippine General Hospital, The Medical City and Cardinal Santos Medical Center. These institutions were selected due to the number of elderly patients who are referred to these hospitals for memory specialist consultation.

Study population and eligibility criteria

Patients who meet the following criteria are eligible for inclusion in the descriptive cross-sectional study:

Inclusion criteria

1. Diagnosed with mild or moderate AD regardless of their treatment status (eg, treatment-naive, already receiving medications or stopped treatment for whatever reason).
2. Age >65 years old.
3. Consulting in one of the three chosen tertiary hospitals in the National Capital Region.
4. Able to read and understand written and spoken English and Filipino.

Exclusion criteria

1. Diagnosed with other types of dementia (eg, vascular dementia, frontotemporal dementia, cognitive impairment with no dementia).
2. Cognitive decline secondary to other deficiencies or abnormalities (eg, vitamin B12 deficiency, thyroid abnormalities, neurosyphilis).

A subset of recruited patients who meet the following inclusion and exclusion criteria will be included further in the exploratory prospective cohort study:

Inclusion criteria

1. Newly diagnosed with mild or moderate dementia using the Montreal Cognitive Assessment-Philippines (MoCA-P)[31] and Clinical Dementia Rating (CDR) scale[32] performed by a licensed psychometrician or neuropsychologist.
2. Clinically diagnosed by an adult neurologist as having probable AD using the NINCDS-ADRDA criteria.[8]

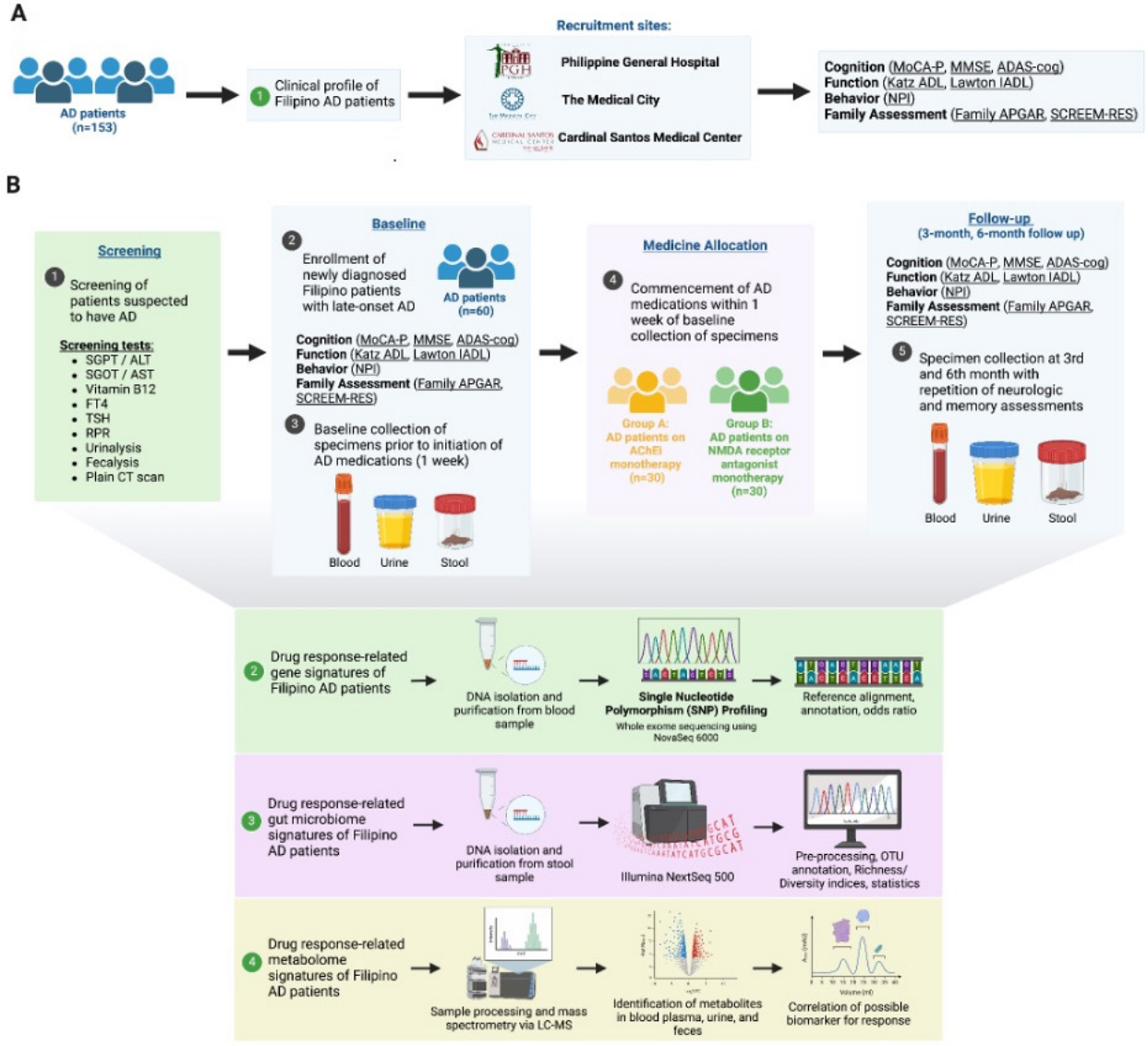

Figure 1 MEMORI-AD workflow. This study will recruit at least 153 patients with dementia aged 65 years old and above. Patients who meet inclusion and exclusion criteria will be enrolled, and their sociodemographic and clinical profiles will be recorded. A subset of recruited AD patients will be followed up for further observation as part of the exploratory prospective cohort to investigate gene, gut microbiome and gut metabolome signatures for treatment response. Patients will be observed from baseline, at 3 month and at 6 month follow-up.

3. Treatment naive for any acetylcholinesterase inhibitors or memantine OR those who have not taken any acetylcholinesterase inhibitors or memantine in the last 3 months for any reason, except for adverse drug reaction;

Exclusion criteria

1. With structural or vascular causes of dementia other than subcortical lacunes (two or less) as seen in plain cranial CT scan.
2. With untreated depression or related psychiatric disorders in the last 6 months.
3. Use of systemic antibiotics in the previous 3 months before providing faecal specimens.
4. Use of corticosteroids, immune-stimulating medications and immunosuppressive agents within the past 2 weeks or those who regularly need them for immune-related disorders;
5. Use of proton-pump inhibitors, H_2-receptor antagonists, tricyclic antidepressants, narcotics, anticholinergic medications, laxatives or anti-diarrheal in the past 4 weeks.
6. Large doses of commercial probiotics consumed ($\geq 10^8$ CFU or organisms per day);
7. Major dietary change during the previous month (defined as eliminating or significantly increasing a major food group).
8. Major gastrointestinal tract surgery in the past 5 years, except cholecystectomy and appendectomy;
9. Major bowel resection at any time.

10. Active uncontrolled gastrointestinal disorders or diseases, including inflammatory bowel disease, indeterminate colitis, irritable bowel syndrome, persistent infectious gastroenteritis, colitis or gastritis, persistent or chronic diarrhoea of unknown aetiology, recurrent *Clostridium difficile* infection, untreated *Helicobacter pylori* infection, and chronic constipation.

These patients will be grouped according to the medications prescribed by their attending physician at baseline: Group A, AD patients given AChE inhibitor monotherapy, and Group B, AD patients given NMDA receptor antagonist monotherapy. The selection, timing and dosage of medications administered to the patients in this study were not influenced in any way by the investigators conducting the research.

Sample size calculation

A minimum sample size of 153 patients will be needed to describe the clinical characteristics of elderly Filipinos with AD (a) based on the study by Dominguez and colleagues[5] where the anticipated proportion of elderly Filipinos with dementia in a cohort in Metro Manila was at 10.6%[5] ; (b) CIs set at 95%; (c) the margin of error set at±5%; (d) the designated effect for random/systematic sampling is 1.0; and (e) non-response set at 10%. The sample size for the exploratory prospective cohort study was determined based on the minimum number needed for PERMANOVA analysis to detect significant differences in multiomics data. A sample size of 30 patients for each group (Groups A and B) for a minimum total of 60 patients will be needed based on pairwise distances and PERMANOVA significance testing[33] to investigate drug response-related genetic, gut microbiome, and metabolome signatures.

Sampling design

The study will employ non-probability purposive sampling among elderly patients consulting for memory problems at the outpatient Adult Neurology clinic of the three study sites. Moreover, a non-probability quota sampling will be implemented for the exploratory cohort study.

Patient recruitment

Patients eligible to participate in the study will be identified through neurologists affiliated with the three tertiary institutions. Study site co-investigators or research associates will contact the patient and/or their legally authorised representative (LAR) to invite them to join. The research team will explain the study's purpose, procedures and voluntary nature, either in English or Filipino. Participants will be informed that they may withdraw at any time without affecting their healthcare. Consent will be obtained for collecting demographics, clinical data, physical exams, laboratory tests, imaging, blood samples and biospecimen banking. Patients will be briefed on potential risks, benefits and compensations, with assurances of privacy and confidentiality. Informed consent, approved by the ethics review board, will be collected via face-to-face, telephone, or video call.

Patients' eligibility to the exploratory cohort study will be confirmed through screening tests using clinical assessment tools, imaging and laboratory tests. Patients will be screened using MoCA-P,[27] CDR scale[32] and with the NINCDS-ADRDA criteria[8] to assess for probable AD. Table 2 shows the diagnostic criteria to be used in the study.

A plain cranial CT scan or a plain cranial MRI/dementia protocol will be performed, if not yet done previously, to confirm the diagnosis of AD and exclude other causes. Other tests consisting of blood chemistry (eg, rapid plasma reagin, serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum vitamin B12, liver function, free thyroxine and thyroid-stimulating hormone tests) will be obtained at the outset if not yet previously done. Serum rapid plasma reagin test, HIV test or cerebrospinal fluid analysis are done on the discretion of the clinician. For this study, only those with probable AD will be accepted and included in the cohort. All other

Table 2 Diagnostic criteria for Alzheimer's disease to be used in the study

Diagnostic tool	Brief criteria
Montreal Cognitive Assessment-Philippines (28)	**Eight domains:** visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall, orientation **Normal:** Score≥21 **Included**: Score of 10–20 inclusive
Clinical Dementia Rating Scale (29)	**Six domains:** memory, orientation, judgement and problem solving, community activities, home and hobbies and personal care **Scoring: 0**, no impairment; **0.5**, questionable; **1**, mild dementia; **2**, moderate; **3**, severe dementia **Included**: Score of 1–2 (mild to moderate dementia)
NINCDS-ADRDA criteria (8)	**Probable AD:** dementia by Mini-Mental State Examination, Blessed Dementia Scale or similar tests *plus* deficits in two or more areas of cognition *plus* progressive worsening of memory *plus* no disturbances of consciousness *plus* age 40–90 years or >65 and an absence of systemic disorders or brain deficits that could account for progressive cognitive decline.

Table 3 Data collection schedule

Procedure	Recruitment and screening	Enrollment/baseline	Month 3*	Month 6*
Eligibility criteria	✓			
Informed consent	✓			
Screening tests	✓*			
Clinical history		✓	✓	✓
Physical and neurologic exam		✓	✓	✓
Neuropsychiatric assessment		✓	✓	✓
Family assessment		✓	✓	✓
Blood collection (10 mL)		✓*	✓	✓
Stool collection		✓*	✓	✓
Urine collection (30 mL)		✓*	✓	✓

*For cohort study only

patients with non-AD dementia will receive appropriate treatment but will no longer be part of the study.

Data collection

Recruited participants will be scheduled for patient examination and will be assessed during enrollment via face-to-face or video conferencing. Patients identified to be eligible for the cohort study will be examined in the clinics at baseline, third month and sixth month of follow-up. Additionally, blood, urine and stool samples will be obtained to determine gene, gut microbiome and metabolome signatures. At baseline, samples will be collected before the initiation of their prescribed treatment. Table 3 summarises the data collection schedule of the study.

Clinical history, physical and neurologic examinations will be conducted by neurology residents, while neuropsychiatric assessments will be administered by a licensed psychometrician or neuropsychologist. The family assessment will be conducted by a family medicine physician. Professional services of a phlebotomist will be sought for home service blood collection from the participants, while patients and their LARs will be instructed on the proper collection and packaging of the urine and stool samples. These specimens will be sent to the study's designated laboratory via a courier contracted by the study group.

Sample collection

The samples that will be collected from the patient are summarised in table 4. The sample collection procedure is detailed in online supplemental file 1. Briefly, at baseline, blood will be collected for screening tests (online supplemental figure 1) and for storage for omics analysis (online supplemental figures 2 and 3). Additionally, urine samples will be obtained both for urinalysis and for omics analysis (online supplemental figure 4). Similarly, stools will be collected for fecalysis and omics analysis using OMNIgene-GUT and OMNImet-GUT (online supplemental figure 5). At month 3 and month 6 follow-up, blood, urine and stool will be collected for the omics analysis.

Specimen analysis

Whole exome profiling

To identify gene signatures specific to AD patients, genomic deoxyribonucleic acid (gDNA) from the isolated buffy coat sample will then be extracted using a DNeasy Blood & Tissue kit (cat. nos. 69504 and 69506). Extracted human gDNA will be sent to the DNA Sequencing Core Facility of the Philippine Genome Center (PGC) for whole exome sequencing using an Illumina NovaSeq 6000 platform (Illumina, CA, USA). Bioinformatic analysis of WES data will be analysed by following the best practices and recommendations of the Core Facility for Bioinformatics, PGC. The association of gene polymorphisms with drug response in AD will be determined and will not be limited to gene polymorphisms previously associated with AD-drug response (online supplemental table 1).

Gut microbiome shotgun metagenomic profiling

To investigate drug response-related gut microbiome signatures among AD patients, a food diary will be completed. Fecal gDNA from the fresh stool samples collected using the OMNIgene•GUT (DNA Genotek, ON, Canada) stool specimen collection kits will be extracted using an QIAamp Powerfecal Pro DNA kit. Extracted fecal gDNA will be sent to the DNA Sequencing Facility of the PGC for gut microbiome shotgun metagenomic sequencing using an Illumina NextSeq 500 platform (Illumina, CA, USA). Bioinformatic analysis and visualisation of microbiome data will be analysed following the best practices and recommendations of the Core Facility for Bioinformatics of the PGC. The association of gut microbiome signatures with drug response in AD will be determined.

Untargeted Metabolomic profiling

To investigate drug response-related metabolome signatures, fecal specimens obtained using OMNImet•GUT

Table 4 Samples to be collected from the participant

Human biological specimens and screening tests	Enrollment/baseline	Month 3	Month 6
4 mL blood (1 pc 4 mL red-top tube) Screening test: ► RPR	 ✓ ✓		
15 mL blood (3 pcs 5 mL gold-top tube) Screening tests: ► SGPT / ALT (KINETIC) ► SGOT / AST (KINETIC) ► Vit B12 (ECLIA) ► FT4 ► TSH	 ✓ ✓ ✓ ✓ ✓ ✓		
30 mL urine for urinalysis	✓		
Fresh stool sample for fecalysis	✓		
8 mL blood (2 pcs 4 mL purple-top tube) for omics analysis	✓	✓	✓
8 mL blood (2 pcs 4 mL red-top tube) for omics analysis	✓	✓	✓
30 mL urine for omics analysis	✓	✓	✓
Stool obtained from OMNIgene•GUT | OM-200 for microbiome analysis	✓	✓	✓
Stool obtained from OMNImet•GUT | ME-200 for metabolomics analysis	✓	✓	✓

ALT, alanine aminotransferase; FT4, free thyroxine; RPR, rapid plasma reagin; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; TSH, thyroid-stimulating hormone.

(DNA Genotek, ON, Canada), the collected blood serum and urine (30 mL) during the aforementioned sampling points will be processed. Sample preparation and extraction methods for faecal samples shall be adapted with modifications from the protocol of Zhang *et al*.[34] Processed samples will be sent to the Protein, Proteomics and Metabolomics Facility of PGC for untargeted metabolomic profiling using liquid chromatography with mass spectrometry (LC-MS). LC-MS will be performed using an RSLCnano 3000 nanoUHPLC system in tandem with an Orbitrap Fusion Tribrid using a heated electrospray ionisation source. Bioinformatics analysis and visualisation of metabolome data will be analysed following the best practices and recommendations of the said facility. Metabolite identification shall be performed in comparison to available reference databases using the mass-to-charge ratio of samples. The association of metabolome signatures with drug response in AD will be determined.

Data collection tools

Clinical history, physical and neurologic examination

To describe the clinical profile of Filipino patients with AD, the Comprehensive Geriatric Assessment for research form[35] will be used to obtain demographics, medical history, family medical history, personal and social history, review of systems and physical and neurologic examination.

Neuropsychiatric assessment Tools

Cognition assessment measure

The cognitive domain will be measured using (a) MoCA-P,[31] (b) Mini-Mental State Examination (MMSE)[36 37] and (c) Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog).[38 39] The MoCA-P is a validated Filipino translated tool consisting of a 30-point questionnaire that assesses the level of cognitive impairment by assessing different domains, namely, visuospatial, naming, memory, attention, language, abstraction, delayed recall and orientation, with lower scores indicating higher levels of cognitive impairment. The MMSE is an 11-item questionnaire assessing orientation, registration, attention and calculation, recall and language and praxis, with a lower score indicative of increased cognitive impairment.[36 37] The ADAS-cog is an 11-item questionnaire that investigates the different cognitive components, including memory, language and praxis, with higher scores indicating increasing levels of dysfunction.[38 39]

Function assessment measure

The function domain will be assessed using (a) Katz Index of Activities of Daily Living (ADL)[40] and (b) The Lawton Instrumental Activities of Daily Living (IADL) Scale.[41] The Katz Index ADL is a six-item questionnaire that assesses the patient's need for assistance in performing basic activities of daily living including bathing, dressing, toileting, transferring, continence and feeding. A score of less than two points indicates severe impairment, four points indicate moderate impairment and six points indicate full function.[41] The Lawton IADL is an eight-item questionnaire that assesses the patient's ability to carry out more complex tasks such as meal preparation, housework, laundry, taking medications, travelling, shopping, use of telephone and handling finances. Scores range

from zero, indicating low function and dependency up, to eight, indicating high functionality and independence.[41]

Behavior assessment measure

The behavioural domain will be evaluated using the Neuropsychiatric Inventory (NPI), a validated informant-based interview that assesses 12 neuropsychiatric symptoms (delusions, hallucinations, agitation/aggression, dysphoria/depression, anxiety, euphoria/elation, apathy/indifference, disinhibition, irritability/lability, aberrant motor, nighttime behaviour and appetite/eating) over the previous month, in terms of frequency, severity and levels of distress with higher scores, indicating more severe behavioural disturbances.[42 43]

Family assessment Tools

The Filipino Family APGAR and SCREEM Family Resources Survey (SCREEM-RES) assessment tools will be used in the study.[44] Filipino family APGAR is used to assess family functioning based on five parameters: adaptability, partnership, growth, affection and resolve. The total scores range from 0 to 10 with higher scores indicating higher levels of satisfaction with family functioning. The SCREEM-RES is a validated tool to measure family resources that the family uses to cope with difficult situations. This instrument is a 12-item questionnaire that explores the social, cultural, religious, education, economic and medical resources of a family. Total scores range from 0 to 36 with higher scores indicating adequate family resources.

Plans for data processing and analysis

Because there is still no cure for AD and the natural course of the disease is that of gradual decline in cognitive function, any intervention that will stabilise or minimally improve the patient's condition is considered a treatment response. This study will adapt the term known as cognition+ where treatment response is considered when there is improvement (ie, ADAS-cog improvement score of at least four points) or no deterioration in cognitive function with evidence of improvement in at least one other measure of function (as measured by at least<1 point decline in Katz ADL score)[45] or behaviour (assessed with a decrease in NPI score of ≥4 points).[46 47]

All data will be recorded as continuous or categorical variables, where appropriate. Entered data will be validated and clarified by a designated data manager from the project staff. All data gathered will be stored and managed electronically using the Research Electronic Data Capture (REDCap) software, which is licensed and administered by the University of the Philippines Los Baños—College of Veterinary Medicine. Raw sequencing reads and metabolomic raw data will be stored and made available to other researchers on reasonable request.

Data will be exported to R software (https://www.r-project.org/) for data processing and statistical analysis. Collected data will be summarised using descriptive statistics, tables and graphs. Means, medians, SD and IQR will be computed for continuous variables, while frequencies and percentages will be obtained from categorical variables. Statistical comparisons between continuous variables will be performed with an independent Student's t-test for normally distributed data, while a Mann–Whitney U test will be used if otherwise. For categorical variables, an χ^2 test or Fisher's exact test will be done. Univariate and multivariate logistic regression will be performed to explore the association of genetic, microbial, and metabolomic signatures with drug response to first-line AD therapeutics. All statistical tests will be two-sided, and a p value <0.05 will be considered statistically significant, following Bonferroni multiple comparison correction.

Genomics

Input raw reads obtained from raw high-throughput sequencing pair-end reads (fastq) will go through computational quality control (QC) and trimming steps using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastq) and Trimmomatic.[48] The alignment of the pair-end sequence reads to a human reference genome build 19 (UCSC hg19) will be done using Burrows–Wheeler Aligner (BWA).[49] Picard (www.picard.sourceforge.net/index.shtml) will be used for the post-alignment processing of the sequence, and the Genome Analysis Toolkit (GATK) will be used for variant calling in sequencing data. Annotating variants will be performed using ANNOVAR, followed by the Variant Effect Predictor for a more reliable classification of the variants. For downstream analysis, visualisation of variants, pathway analysis and linking variants and drugs will be performed using integrative genomics viewer, KEGG, and PharmGKB, respectively, to determine the drug-response related gene signatures from each sample.

Metagenomics

Technical sequences obtained from raw Illumina MiSeq sequencing pair-end reads will undergo computational QC and trimming steps using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastq) and Trimmomatic.[48] Two steps will be performed: (1) taxonomic profiling, including taxonomic classification using Metagenomic Phylogenetic Analysis (MetaPhlAn2), visualisation of the phylogenetic tree of the taxonomic profiles using Graphical Phylogenetic Analysis (GraPhlAn), followed by the diversity analysis in accordance with the taxonomic composition of gut microbiota by measuring the Shannon index using vegan R package.[50] The other one is (2) functional profiling using The HMP Unified Metabolic Analysis Network 2 (HUMAnN2) to identify the gene profiles for gut microbiome functions. For the statistical analysis, the dataset will undergo Kruskal–Wallis H test using Statistical Analysis of Metagenomic Profiles (STAMP) to detect the significant difference among taxonomic and functional features of the metagenomic profiles among the study groups.

Metabolomics

Raw data will be processed using the pre-set processing Workflow tree in the Compound Discoverer 3.2, 'Untargeted Metabolomics with Statistics Detect Unknowns with Mapped Pathways and ID using Online Database' and will be utilized as a framework for the analysis. Replacing missing values with substituted data will be performed using a Random Forest Algorithm. Afterwards, molecular networks will be analysed to determine associations between the matching fragmentation spectra. Relevant information about each of the detected compounds based on its corresponding data such as chromatograms, mass spectra, results from database searching and library matching, and the univariate statistical test will then be analysed using Metabolika Pathways to determine the significant biochemical pathways involved via biochemical pathway mapping. The mzCloud search will be performed as the main database for putative metabolite identification.

Ethics and dissemination

The study has received ethical clearance from the Department of Health Single Joint Research Ethics Board (SJREB-2022–15). All ethical principles in the conduct of scientific research, as well as national laws and regulations, will be adhered to at all times throughout the conduct of this project. Informed consent will be obtained from the participant or his/her LAR as described above. The potential participant/LAR will be informed of the minimal risk of phlebotomy, including haematoma formation. Adverse effects that will arise during or after blood collection will be handled immediately by a licensed physician.

All patients will be given the standard of care or appropriate treatment for their current condition. Other than those specified in the inclusion/exclusion criteria, there will be no discrimination on gender, ethnicity and economic status, with fairness of treatment without prejudice on all participants. Participants and/or their LAR will be informed that they can withdraw anytime without any penalty or any ill effects regarding their current disease management. Participants will receive tokens and monetary compensation for their participation in the study.

The study will comply with the Data Privacy Act of the Philippines 2012. All records and information about the participants will remain confidential, except for the purpose of the study. Electronic data collection forms will be coded and password-protected with access limited to the study proponents. Consent will be valid until the end of the study. In case informed consent is withdrawn, patient data will be irretrievably unlinked from their source by the destruction of all identifiers.

Residual human biological samples after the end of the study will be stored and biobanked for ten years at the College of Medicine, University of the Philippines Manila, under the stewardship of Dr. Fresthel Climacosa. Biobank samples may be used for future research approved by a Philippine Health Research Ethics Board-accredited institutional review board, related or not related to the current study. The identification of the participant in the biobank specimen will remain confidential, and only the current study team will have access to these personal data. When clinically relevant information is revealed in future research, the participant will be informed of the result through their physician. All remaining human biological specimens will be destroyed after ten years of storage and will be disposed of accordingly.

Results of psychometric scales will be made available to enrolled patients. The study results will be presented in national/international conferences and published in international peer-reviewed scientific journals, and summaries of the results will be provided to the study funders, and institutional review boards of the three tertiary referral hospitals.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Department of Health Single Joint Research Ethics Board (SJREB-2022-15). Participants gave informed consent to participate in the study before taking part.

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