Statistical Analysis Plan

for

Koori Growing Old Well Study (KGOWS)

The interaction between apolipoprotein-E genotype and life-course cardiometabolic factors on cognitive decline in older Aboriginal Australians.

SAP Version Number: 1.0

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Contents

1 ADMINISTRATIVE INFORMATION

Statistical Analysis Protocol

Version history

Version	Date	Description
SAP Version 1	25th March 2021	Final version

List of contributors

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2 SIGNATURE PAGE

We, the undersigned, have reviewed and approve this Statistical Analysis Plan.

The signatories confirm that:

a) They believe the procedures for the statistical analysis of the data from the *Koori Growing Old Well* study described in this document are appropriate;
b) Their intention is to analyse the data from the *Koori Growing Old Well* study using the statistical procedures described in this document; and
c) If, subsequently, the statistical analysis of the data is conducted in a way that differs importantly from the procedures described in this document, those differences will be explicitly outlined in reports of those analyses.

Signature  Date

25th March 2021

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25th March 2021

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3. INTRODUCTION

Around the world the prevalence of dementia is increasing and is projected to triple by 2050 [1]. Despite widespread efforts to elucidate the pathological mechanisms of this group of neurodegenerative diseases, efficacious treatments remain limited [2]. In this context, with a view to delaying dementia onset, many researchers have shifted their focus toward the identification of modifiable life-course risk factors. In the 2017 Lancet commission into *"dementia intervention, prevention and care"*, a life-course model indicated that up to a third of dementia cases globally may be attributed to nine modifiable life course factors, two thirds of which were cardiometabolic (i.e., hypertension, obesity, diabetes, physical inactivity and smoking) [3].

Complimenting these findings has been an increasing awareness that individual dementia risk is defined by environmental-genetic interactions [4]. The *APOE*-ε4 allele (which codes for a 34kDa protein - *APOE*) is recognised as one of the strongest genetic risk factors for dementia and particularly Alzheimer's disease [5]. *APOE* is expressed throughout the body and has an essential role in regulating lipid transport and homeostasis, glucose metabolism and cerebrovascular function [6]. Based on this, it has been strongly associated with cardiovascular and metabolic health. In this context there has been a growing understanding that the risk lifetime cardiometabolic health poses to late-life cognition may be strongly influenced by an individual's *APOE* genotype [7].

Aboriginal Australians face some of the highest rates of dementia in the world and a prevalence rate 3-5 times that of the general Australian population [8, 9]. In a study of disease burden in Aboriginal Australians, preventable chronic diseases including cardiovascular disease (CVD) and type 2 diabetes, accounted for up to 70% of the difference in disease burden between Aboriginal Australians and the general Australian population [10]. In combination with this, the frequency of the *APOE*-ε4 allele was found to be significantly higher in Indigenous peoples in Australia compared to those of European descent, and was associated with significant variation in triglyceride and cholesterol concentrations [11]. Despite the burden of cardiovascular disease and dementia, no study has investigated the effect of *APOE* genotype on the association between cardiometabolic risk factors and cognitive decline in an Aboriginal Australian population.

4. STUDY AIMS AND METHODS

This study aims to understand the interaction between cardiometabolic risk factors and *APOE* genotype on cognitive decline in a population-based sample of urban and regional Aboriginal Australians aged 60 years and older. In particular, this study aims to investigate whether *APOE* genotype has a modifying effect on the relationship between lifetime cardiometabolic factors (i.e., diabetes mellitus, treated and untreated hypertension, heart disease, hypercholesterolaemia, obesity, waist-to-hip ratio, cerebrovascular disease) and cognitive decline over 6 years in older Aboriginal Australians aged 60 years and older.

Participants were part of the longitudinal Koori Growing Old Well Study (KGOWS), a population-based study spanning five Aboriginal communities in New South Wales (NSW), Australia with two metropolitan Sydney sites and three regional mid-North Coast sites. The baseline study has been described elsewhere, including dementia prevalence rates and cross-

sectional factors associated with dementia [12-15]. Baseline data collection occurred from March 2010 to September 2012 (n=336). Baseline participants who were contactable and willing to participate were recruited for a follow-up study, with follow-up data collection occurring from July 2016 to April 2018 (n=165).

All follow-up participants were invited to provide a saliva sample for genomic analysis, following prior community consultation indicating majority preference to include this component [16].

The study was approved by the Aboriginal Health and Medical Research Council (AHMRC; 615/07), the University of New South Wales Human Research Ethics Committee (HREC 08003), and NSW Population & Health Services Research Ethics Committee (AU RED Ref: HREC/09/CIPHS/65; Cancer Institute NSW Ref: 2009/10/187).

5. STUDY POPULATION AND SUBGROUPS

Baseline inclusion criteria were identifying as an Aboriginal and/or Torres Strait Islander person, aged 60 years or older, and having resided in one of the five study catchment areas for at least 6 months. All baseline participants were eligible to take part in the follow-up study.

At baseline, dementia screening instruments included the Mini-Mental State Examination (MMSE) [17]; the modified Kimberley Indigenous Cognitive Assessment (mKICA [13]; and the Rowland Universal Dementia Assessment Scale (RUDAS) [18]. At baseline, all participants who screened positive for cognitive impairment (≤26 on the MMSE, ≤35 on the mKICA, and/or ≤25 on the RUDAS) and a 20% random sample of those who screened negative, proceeded to medical/cognitive assessment and contact person interview. At follow-up, based upon validation of the MMSE and mKICA [8], participants who screened positive for cognitive impairment (<26 on the MMSE and/or <37 on the mKICA) proceeded to medical/cognitive assessment and contact person interview.

Diagnosis of dementia and cognitive impairment were based on comprehensive medical data, with assessment by specialist geriatricians or general physicians experienced in dementia assessment; cognitive, neurological and behavioural measures, as well as contact person interview were used for diagnosis. “All-cause” dementia or probable/possible Alzheimer’s disease (AD) were diagnosed according to National Institute on Aging and Alzheimer’s Association (NIA-AA) [19] and DSM-IV diagnostic criteria. Other types of dementia were diagnosed according to available criteria and detailed in previous reports [14]. MCI diagnosis was based on criteria of the International Working Group on Mild Cognitive Impairment [20].

For participants unable to complete a medical assessment at follow-up, evidence of decline was based on changes to cognitive screening test scores from baseline to follow-up. Participants were categorised as having MCI if they experienced a 2SD drop (i.e., ≥3 points) in MMSE score (based on published norms for intact participants of the baseline study) [8] from baseline to follow-up and had no evidence of functional impairment [21]. Participants with an MMSE score <22 and evidence of functional impairment were diagnosed with dementia, consistent with baseline procedure [14].

6. STATISTICAL ANALYSIS

The primary analyses will be conducted using binary logistic regression analyses. All tests will be 2-tailed and the nominal level of α will be 5%. Where data are missing for outcome measures, the number of observations will be reported; no imputation of missing values for the primary outcome will be carried out. P-values will not be adjusted for multiple comparisons; however, outcomes are clearly categorised by importance (i.e., primary and secondary). P-values will be rounded to three decimal places and values less than 0.001 will be reported as <.001.

6.1 Primary Outcomes

Incident cognitive decline is the primary outcome measure. Incident cognitive decline is defined as shifting to a more severe diagnostic category at follow-up (i.e., intact to MCI/dementia, MCI to dementia, or other cognitive impairment to dementia Participants will be categorised as not having declined if their diagnosis remained stable from baseline to follow-up, or they reverted from baseline MCI diagnosis to intact at follow-up.). Participants with baseline dementia will be excluded from analyses.

6.2 Secondary Outcomes

Change in MMSE score from baseline to follow-up will be used as the secondary outcome measure. This score was chosen on the basis of a previous study which found the MMSE to be the most sensitive cognitive screening instrument within this population [22].

6.3 Additional Measures

Demographic data were collected at baseline, including information on age, sex, urban/regional location, educational attainment and work history. Comprehensive life-course and medical history was also taken (see Table 1 for list of measures).

Continuous predictor variables will be standardised (z-scored) prior to inclusion in models.

Table 1. *List of baseline measures to be used as predictor variables in statistical analyses.*

Measure	**Details**
Age	Continuous
Sex	1=female, 2=male
Urban/regional	0=regional, 1=urban
Educational attainment	Continuous
Unskilled work history	0=no, 1=yes
Smoking (pack year history)	Continuous
Smoking status	0=never smoked, 1=ex-smoker, 2=current smoker
Diabetes	0=no, 1=yes
Hypercholesterolemia	0=no, 1=yes
Statin use	0=no, 1=yes
Hypertension	0=no, 1=yes
Body mass index	Continuous
Waist-hip ratio	Continuous
Heart disease	0=no, 1=yes
Stroke/TIA	0=no, 1=yes
APOE genotype (ε2ε2, ε2ε3, ε3ε3 ε2ε4, ε3ε4 or ε4ε4)	0= no ε4 allele, 1= ε4 heterozygous/homozygous
Cardiovascular risk score	Continuous

A cardiovascular risk score will be calculated based on the Framingham Risk score which includes patients age, HDL cholesterol level, Total cholesterol, treated or untreated systolic blood pressure, smoking and diabetes status. As serum cholesterol levels were not measured in this cohort, proxy values will be allocated to those who self-reported high cholesterol. These values are based on clinical cut offs (Table 2.) [23, 24].

Table 2. *Serum cholesterol clinical guidelines*

	Total cholesterol (mmol/L)	**HDL cholesterol (mmol/L)**
Low	n/a	<40
Good	<200	>/= 60
Moderately elevated	200-239	n/a
High	>/= 240	>/= 60

Abbreviations: HDL, high density lipoprotein.

6.4 Analyses of Primary Outcome

Descriptive statistics for continuous (mean, standard deviation, median, interquartile range, range) and categorical/dichotomous variables (number and percentage in each group) will be reported (for both original and imputed data).

Univariable comparisons will be made between groups (incident cognitive decline vs no decline) using logistic regression analyses for all predictor variables. These analyses will be adjusted for age and follow-up-time (entered as covariates). Each cardiometabolic predictor will also be entered into separate moderator analysis whereby multivariable logistic regression models will be run with cognitive decline as the outcome and *APOE* genotype, the respective cardiometabolic factor (e.g., obesity) and an interaction term of *APOE**cardiometabolic factor as predictors.

6.5 Analyses of secondary outcomes

Previous research has shown MMSE to be a reliable screening measure for cognitive decline in this study population [22]. All models will be run a second time with MMSE scores used as a continuous outcome measure of cognitive decline.

6.6 Additional Analyses

If no effects of cardiovascular/metabolic risk factors are seen (specifically: hypertension, hypercholesterolemia, diabetes, heart disease), sensitivity analyses will be conducted examining age of diagnosis for these factors, to tease apart potential reasons for lack of effects such as mid versus late-life onset and the impact of chronic disease management.

6.7 Missing Data and Outliers

Multiple imputation will be conducted for predictor variables prior to conducting analyses. Cognitive outcomes (i.e., MMSE score and cognitive decline) and APOE variables will be included as indicators in imputation but will not have missing values replaced.
Cardiovascular risk scores will not be imputed but will be calculated following imputation of individual variables that make up the Framingham cardiovascular index.

If outliers are present in the data or the model assumptions are grossly violated, sensitivity analyses will be conducted for those specific models.

10. RATIONALE FOR ANY DEVIATION FROM PRE-SPECIFIED ANALYSIS PLAN

Logistic regression analyses will not be conducted if the following criteria is not met: minimum of 5 events per variable for logistic regression analyses [25]. Instead, in the event of separation or quasi-separation, either Firth's penalised regression for rare events procedure [26] or Bayesian logistic regression will be conducted depending on appropriateness.

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