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**Brief Report Title:** Testing for interactions between *APOE* and *Klotho* genotypes on cognitive, dementia and brain imaging metrics in UK Biobank.

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Abstract

Recent research suggests genetic variation in the *Klotho* locus may modify the association between *APOE* e4 and cognitive impairment. We tested for associations and interactions between these genotypes vs. risk of dementia, cognitive abilities, and brain structure in older UK Biobank participants. *Klotho* status was indexed with rs9536314 heterozygosity (vs. not), in unrelated people with vs. without *APOE* e4 genotype, corrected for various confounders. *APOE* e4 associated with increased risk of dementia, worse cognitive abilities and brain structure. *Klotho* was associated with better reasoning. There were no interactions; potentially suggesting an age- and pathology-dependent *Klotho* effect.

Key points

- **Question:** *Klotho* genotype has been previously shown to 'offset' a substantial amount of the *APOE* e4/cognitive impairment association. Is this modification effect apparent in large-scale independent data, in terms of non-demented cognitive abilities, brain structure and dementia prevalence?

- **Findings:** In aged 60 years and above participants from UK Biobank, we found significant associations of *APOE* and *Klotho* genotypes on cognitive, structural brain and dementia outcomes, but no significant interactions.

- **Meaning:** This could reflect somewhat healthy participants, prior type 1 error or cognitive/dementia ascertainment imprecision, and/or that *Klotho* genotypic effects are age and neuropathology dependent.
Introduction

Preserving cognitive abilities such as memory is a common concern into older age, and in the absence of reliable treatments, the public health priority is prevention and delay of cognitive impairment\(^1\), including understanding effect modifiers. APOE e4 is a known risk factor for AD and cognitive decline\(^1\). Genetic variation in the KL locus has been associated with ageing-related phenotypes including insulin resistance and brain function\(^2\). A recent study of AD cohorts, longitudinal conversion and amyloid-beta samples showed a statistically significant ‘modification’ effect where the deleterious effects conferred by APOE e4 were offset by heterozygosity based on two KL polymorphisms in strong linkage disequilibrium: F352V (rs9536314) and C370S (rs9527025), possibly due to correlations with increased serum Klotho\(^3\). UK Biobank is a relatively large general population cohort\(^4\) where we have previously shown deleterious effects of APOE e4 on cognitive\(^5\), structural brain imaging\(^6\) and AD/dementia phenotypes\(^7\). This brief report tested the hypothesis that based on recent research, genetic KL variation would interact with APOE e4 genotype in relevant cognitive, brain and dementia phenotypes.

Methodology

Study design and participants

UK Biobank is a prospective cohort study including 502,628 participants who attended one of 22 baseline assessment centres from 2006 to 2010, aged 40-70 years\(^8\). In 2014, MRI scanning of a sub-group of 100,000 participants began, and this is ongoing. This project was completed using UK Biobank application #17689 except for the hospital admission and episode (HES) analyses which were conducted using project #7155.

Ethical approval and data availability

This analysis was conducted under generic approval from the NHS National Research Ethics Service (approval letter dated 17th June 2011, ref 11/NW/0382). Written informed consent was obtained from all participants in the study.
Dementia outcomes

Dementia/AD outcomes were generated in two ways: firstly, derived by UK Biobank using self-report, hospital admission and death record data, with data utilising International Classification of Diseases version 10 (ICD-10 codes). Individuals were designated as cases (“all-cause dementia” or “Alzheimer disease”) if they had indicated either in self-report or hospital/death records – derived by UK Biobank\(^7,9\). Those coded as missing were designated as controls (i.e. did not self-report dementia, and diagnoses not present in hospital/death records). The UK Biobank-derived ascertained cases were the latest available as of October 2020. Secondly we supplemented this with record-based hospital admission/episode (HES) data using open-access methods described previously\(^10\) where dementia was defined as ICD-10 codes F00 (dementia in Alzheimer disease), F01 (vascular dementia), F02 (dementia in other diseases), or F03 (unspecified dementia); hospital admission records were available until February 2018 for the full cohort, whereas linkage to primary care records was available for 45% of the UK Biobank cohort (approximately 230 000 participants) until May 2017, for Scotland, September 2017, for Wales, and August 2017 for England.

Imaging data

The release of brain MRI data as of July 2020 was used (i.e. approximately 40k). All imaging data used here was processed and quality checked by UK Biobank\(^11\). We selected imaging phenotypes \textit{a priori} shown to be associated with worse cognitive ability and decline total white and grey volumes adjusted for skull size (WM/GM respectively); log WM hyperintensity volume (WMH); overall hippocampal volume; general factors of fractional anisotropy (FA) and mean diffusivity (MD), and frontal lobe GM (gFrontal)\(^12\) based on principal components analysis (PCA). Total WM hyperintensity volumes were calculated based on T1 and T2 fluid-attenuated inversion recovery (FLAIR), derived by UK Biobank.

Cognitive data
Five tests were completed at baseline (2006-2010), of which we examine three here which have shown sufficient intra-participant reliabilities: Pairs-matching 6-pair (memory), verbal-numeric reasoning and log reaction time (processing speed). We also examined four cognitive tests administered from 2014 onwards. These were: Trail making test a+b (processing speed/executive function) and Digit symbol substitution (executive function) assessed via online follow-up, plus Matrix pattern completion (nonverbal reasoning) and Tower rearranging (executive function) at MRI.

Genetic data
UK Biobank genotyping was conducted by Affymetrix using a bespoke BiLEVE Axiom array for ~50,000 participants and the remaining ~450,000 on the Affymetrix UK Biobank Axiom array. All genetic data were quality controlled by UK Biobank as described by the protocol paper. APOE e4 ‘risk’ genotype presence (vs. non-e4) was genotyped based on rs7412 and rs429358. KL was indexed using rs9536314 where G/T is considered protective (vs. G/G; T/T) and synonymous with KL-VS diplotype heterozygosity.

Covariates
Participants self-reported their smoking history: current, past or never, medication use for dyslipidaemia, hormone replacement therapy, blood pressure, oral contraceptive or insulin. We excluded participants for whom these data were missing (<5%). Townsend deprivation indices were derived from postcode of residence.

Statistical analysis
PLINK v1.90 was used for genetic quality controlling and Stata V.14 was used for statistical analyses. We removed participants who reported neurological conditions as described previously. We statistically controlled for: age, sex, Townsend, ever-smoking, genotypic array, baseline/MRI assessment centre, 8 principal components, array, and medication (concurrent to the phenotype under study; dementia outcomes used baseline values). We
focussed on participants aged ≥60 years at baseline or imaging (respectively for those analyses). We excluded participants with non-white British ancestry, self-report vs. genetic sex mismatch, putative sex chromosomal aneuploidy and excess heterozygosity. In terms of quality controlling we accounted for relatedness between participants by removing one random participant in cases where two individuals were 2nd cousins or closer, and this was based on central UK Biobank-derived relatedness coefficients (https://biobank.ndph.ox.ac.uk/ukb/label.cgi?id=263). We included polymorphisms in Hardy Weinberg equilibrium (P > 1*10⁻⁶), polymorphisms with missingness rate less than 0.1 and minor allele frequency> 0.01 and imputation score > 0.8. We have previously reported power calculations indicative of >95% confidence to find ‘true’ effect sizes at Cohen’s D = 0.1 (i.e. small) with regard to APOE genotype and outcomes in UK Biobank. Multiple power calculations using G*Power 2, for a ‘true’ effect at Cohen’s D = 0.1 (where 0.2 is considered a small effect size) at p=0.05, estimated the least power to find a ‘true’ effect was in the imaging sample, with 96% estimated power post-hoc based on current group sample sizes.

Results

Descriptives

After exclusions there were baseline N=169,374 (mean age 64.1, SD=2.89) participants; imaging n=26,903 (68.14 years; SD 5.02). Allele frequencies for baseline/imaging analyses are shown in Supplementary Table 1. There were n=1,570 UKB-ascertained dementia cases (0.9%) of which n=634 were AD (0.4%). There were n=3,346 (1.9%) dementia cases based on HES of which n=1,577 (0.9%) were AD.

Outcomes

Supplementary Table 2 shows associations between APOE e4 genotype and multiple worse outcomes: UK Biobank-ascertained dementia (odds ratio[OR]=3.27 for e4 vs. not) and AD (OR=5.06), HES-based dementia (OR = 3.27) and AD (OR=5.06), cognitive scores on Matrix Completion (-0.04 SDs for e4 vs. not), frontal lobe GM (-0.05), hippocampal volume (-0.06),
log TMT total time (0.037) and Digit symbol (-0.075; significant P-value range <0.001 to 0.038).

KL heterozygosity (vs. not) was associated with better reasoning only (0.021SDs; P=0.042).

There were no statistically significant APOE/KL interactions.

As sensitivity analyses all models were re-run: unadjusted then covariates added incrementally; excluding people with concurrent neurological conditions13; testing a dose effect of 0/1/2 G allele copies (i.e. including an extra n=4,489 with GG) rather than the primary T/T vs. G/T test throughout, and using the full sample (aged <60). These made no difference to the results.

Discussion

A recent study by Belloy et al.3 reported a protective modifying effect of KL heterozygosity on APOE e4 genotype’s conferred risk on cognitive impairment and dementia, in a collation of longitudinal, AD and amyloid-beta cohorts totalling N=24,743. Using unrelated UK Biobank data we tested whether a similar effect could be seen in multiple outcomes: AD/all-cause dementia vs. not, and non-demented cognitive and structural brain MRI phenotypes known to underlie cognitive decline. Participants were ≥60 years as per Belloy et al. We identified individual APOE and KL genotype/outcome associations but no interactions, against our hypothesis. There could be an underestimation of true effect due to cognitive test imprecision13 or generally preserved participant health12. No interaction here vs. Belloy et al. could reflect the use of different phenotypes: the original study investigated AD case vs. control status, conversion to impairment and amyloid-beta while this study investigated AD status and non-demented cognitive/brain structure values. UK Biobank derived dementia status largely from ICD codes whereas Belloy et al. used clinical and/or pathological ascertainment. This could suggest that the age-dependent changes in KL expression, and interaction with APOE status, manifest only beyond at least moderate AD-related neuropathology (e.g. amyloid or tau)15. The null AD case/control interaction could reflect that UK Biobank is relatively healthy and well-educated. It is possible the UK Biobank participants were not sufficiently old; analysis of
longitudinal cognitive and brain imaging data (in independent data) is indicative of more pronounced KL heterozygosity effects particularly into later life\textsuperscript{16}. By contrast our findings are more in-line with de Vries et al.\textsuperscript{16} who reported protective rather than deleterious effects of KL heterozygosity on longitudinal cognitive decline.

**Limitations**

This study did not explore an exhaustive list of structural imaging phenotypes. There is some degree of healthy volunteer bias in UK Biobank participants, and probably more so in participants who returned for imaging. Some imaging participants would have been unable to complete scanning due to contraindications related to poorer health, e.g. pacemakers/stents\textsuperscript{12}. Dementia ascertainment was based on a mixture of self-report, HES and death data; participants did not have regular cognitive assessment e.g. with Mini-Mental State Exam, and hence there may be some degree of underestimation of dementia in the current data.

**Summary**

A recent relatively large-scale study including cohort, case/control longitudinal and amyloid-beta data showed a significant interaction whereby KL genotype modified the well-known APOE e4 and dementia association. Using independent cognitive, structural brain and dementia data, we did not support these prior findings; this could reflect some degree of bias or imprecision in UK Biobank participants or phenotypes, or that the interaction while ‘true’ is contingent on AD-related neuropathology: future studies should investigate this further in deeply-phenotyped cohorts.

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Drafting of the manuscript: DML, RT.
Critical revision of the manuscript for important intellectual content: All co-authors.
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