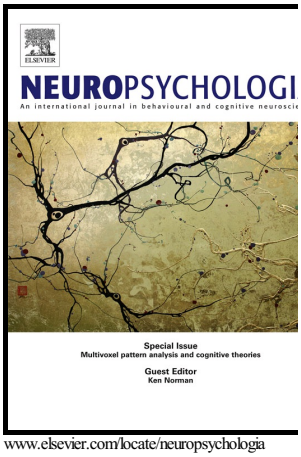

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Investigating the influence of *KIBRA* and *CLSTN2* genetic polymorphisms on cross-sectional and longitudinal measures of memory performance and hippocampal volume in older individuals

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Abstract

The variability of episodic memory decline and hippocampal atrophy observed with increasing age may partly be explained by genetic factors. *KIBRA* (kidney and brain expressed protein) and *CLSTN2* (calsyntenin 2) are two candidate genes previously linked to episodic memory performance and volume of the hippocampus, a key memory structure. However, whether polymorphisms in these two genes also influence age-related longitudinal memory decline and hippocampal atrophy is still unknown. Using data from two independent cohorts, the Sydney Memory and Ageing Study and the Older Australian Twins Study, we investigated whether the *KIBRA* and *CLSTN2* genetic polymorphisms (rs17070145, rs6439886) are associated with episodic memory performance and hippocampal volume in older adults (65-90 years at baseline). We were able to examine these polymorphisms in relation to memory and hippocampal volume using cross-sectional data and, more importantly, also using longitudinal data (2 years between testing occasions). Overall we did not find support for an association of *KIBRA* either alone or in combination with *CLSTN2* with memory performance or hippocampal volume, nor did variation in these genes influence longitudinal memory decline or hippocampal atrophy in two cohorts of older adults.

Key words: *KIBRA*; *CLSTN2*; gene-gene interactions, episodic memory, hippocampus

1. Introduction

Episodic memory declines with age (Rönnlund et al., 2005) and yet our current understanding of the underlying biological processes is rudimentary. Heritability estimates of 30-60% for memory suggest that genetics plays a major role in memory performance (McClearn et al., 1997; Papassotiropoulos & de Quervain, 2011). Despite significant heritability, only a small number of genes have consistently been associated with memory performance and the diversity of the results is striking. The first GWAS of episodic memory function resulted in the identification of *KIBRA*, kidney and brain expressed protein (also known as WW and C2 domain containing 1, *WWC1*), as a candidate gene affecting memory performance as well as functional brain activity based on three separate cohorts within the age range of 18-81 years (Papassotiropoulos et al., 2006). From this study a single nucleotide polymorphism (SNP) leading to a substitution of C → T (rs17070145) in *KIBRA* was identified which positively affects memory performance. Further, it was shown that non T-carriers over-recruit hippocampal regions during episodic memory retrieval. Following this initial study, several studies have replicated the findings in samples with ages between 30 – 89 years (e.g. Almeida et al., 2008; Schaper, Kolsch, Popp, Wagner, & Jessen, 2008; Vassos et al., 2010), although the results have not always been consistent using similar samples ranging in age from 18-77 years (e.g. Nacmias et al., 2008, Need et al., 2008). For example, in a large-scale attempt to replicate the association of the *KIBRA* SNP to episodic memory, including participants between 35 – 85 years of age, Kauppi and colleagues (2011) confirmed that the T-allele was associated with improved memory performance when using an immediate free recall word task, an effect that was augmented with increasing age. However, in contrast with the original findings, T allele carriers showed increased hippocampal activity instead of the non-carriers. There are also other studies that have failed to find any association with the *KIBRA* SNP and memory (e.g. Need et al., 2008). One study even found that carriers of the T-allele performed

more poorly on long-term memory tests (Nacmias et al., 2008) and that the T-allele is associated with increased risk of developing Alzheimer's disease (Rodríguez-Rodríguez et al., 2009). Even when examining past studies that exclusively investigated the effect in older samples (>50 years of age) the results are mixed with studies in favor of the positive association of *KIBRA* T-allele and episodic memory (Almeida et al., 2008; Schaper et al., 2008) and against such association (Nacmias et al., 2008; Rodríguez-Rodríguez et al., 2009). Moreover, *KIBRA*-status was not able to predict whether individuals would decline or maintain episodic memory function over 15 years (Josefsson et al., 2012). Thus, the effect of the *KIBRA* SNP, rs17070145, on memory performance is still unclear, as indicated by a recent meta-analysis (Milnik et al., 2012) and review (Schwab et al., 2014).

Another polymorphism identified by the Papassotiropoulos et al. (2006) memory GWAS was a *CLSTN2* (calsyntenin 2) SNP (rs6439886), however, this association was not confirmed in the replication sample. Subsequently this association has been replicated in two other independent studies in both adolescents and older adults (Jacobsen, Picciotto, Heath, Mencl, & Gelernter, 2009; Laukka et al., 2013).

The mechanism/s by which these two polymorphisms (*KIBRA* rs17070145, *CLSTN2* rs6439886) may influence episodic memory functioning is unclear. *KIBRA* encodes a phosphoprotein, which binds to many proteins including those implicated in neuronal and synaptic plasticity, cell migration, vesicular transport, mitosis and tumorigenesis (Zhang et al., 2014). It has been proposed that *KIBRA* regulates AMPA receptors, the major excitatory synaptic receptors of the brain, suggesting KIBRA may be involved in synaptic plasticity and transmission (Makuch et al., 2011; Zhang et al., 2014). *CLSTN2* is part of the components of the postsynaptic membrane and is located predominately in excitatory synapses giving *CLSTN2* a role in intracellular postsynaptic signaling, potentially mediating specific responses in excitatory synaptic transmission (Hintsch et al., 2002).

Interactions between genes may also contribute to memory performance. However, few studies have appraised gene-gene interactions and memory performance. In 2010, Preuschhof et al. observed an interaction between two SNPs, located in the *KIBRA* and *CLSTN2* genes (rs17070145, rs6439886), on episodic memory performance in young adults. Individuals who were both *KIBRA* T and *CLSTN2* C-carriers had the highest performance compared to *KIBRA* C homozygotes/*CLSTN2* C-carriers who had the lowest performance. However, a recent study failed to replicate previous results (Sédille-Mostafaie et al., 2012).

One major limitation with previous attempts to understand the effects of *KIBRA* or *CLSTN2* on memory is that prior investigations have been cross-sectional. Notably, it has been suggested that the genetic influence on cognition is magnified at older ages (Lindenberger et al., 2008; Papenberg et al., 2013) as well as playing an important role in the cognitive changes associated with increased age (Mattay, Goldberg, Sambataro, & Weinberger, 2008). This implies that it is important to investigate cognitive change over time in relation to specific genetic polymorphisms in order to better understand the complex relationships between genetics and age-related memory performance.

Both *KIBRA* and *CLSTN2* are highly expressed in the medial temporal lobe (Zhang et al., 2014; Schneider et al., 2010; Hintsch et al., 2002), a brain region important for episodic memory (Squire, Stark, & Clark, 2004). Moreover, it has been shown that in the Older Australian Twins Study and other cohorts that ~53-65 % of the variance in hippocampal volume can be attributed to genetic influences (Blokland, de Zubicaray, McMahon, & Wright, 2012; Mather et al., 2015). In the original paper by Papassotiropoulos et al. (2006) there was no *KIBRA* genotype difference in hippocampal volume. However, a recent study by (Palombo et al., 2013) showed that *KIBRA* T-allele carriers had larger total

hippocampal volume compared to non-carriers. It should be noted that both these studies investigated younger participants, were cross-sectional and used small sample sizes (N≤32). Thus, the association between certain memory-related genetic polymorphisms, such as *KIBRA* and *CLSTN2*, and memory-related brain structures, such as the hippocampus, should also be investigated in larger samples using older participants and measuring longitudinal change.

The aim of the present study was to investigate the effect of these previously described *KIBRA* and *CLSTN2* polymorphisms and their interaction on memory performance both cross-sectionally and longitudinally in two independent studies of older adults. Additionally, we aimed to assess the effect of these SNPs on hippocampal volume and hippocampal atrophy.

2. Method

2.1. Samples

2.1.1. Sydney MAS

The Sydney Memory and Ageing Study (Sydney MAS) is a longitudinal community-based study of non-demented older adults aged 70-90 years at baseline (Wave 1). Participants were excluded if they had a previous diagnosis of dementia, schizophrenia or bipolar disorder, multiple sclerosis, motor neuron disease, developmental disability, progressive malignancy or any other medical or psychological conditions that may prevent from completing the assessments; if they scored <24 on the Mini-mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975) adjusted for education, age and non-English speaking background (Anderson et al., 2007). A detailed interview, including neuropsychological assessment, is undertaken every two years. For more details see Sachdev et al. (2010). Ethics approval from the appropriate committees was granted and each participant provided written informed consent to participate. For the present study only those participants with genotyping data

available for the *KIBRA* and *CLSTN2* SNPs and with an adjusted MMSE score > 24 were included in the analysis giving a maximum sample size of 912 individuals (508 women) for neuropsychological performance at Wave 1 and 805 individuals at Wave 2 (435 women). Within this sample, structural MRIs were available from 501 individuals (279 women) at Wave 1 and 327 individuals (174 women) at Wave 2. The time interval between Waves was 2 years. The age of the participants were distributed accordingly: 32.6 % were 70-75 years; 35.0 % were 76-80 years; 24.8 % were 81-85 years; and 7.6 % were 86-90 years of age. See Table 1 (full sample) and Table 2 (MRI-sample) for demographic data including age, education, and health variables split by the *KIBRA* polymorphism. See supplementary Table S1 (full sample) and S2 (MRI-sample) for demographic information split by the *CLSTN2* polymorphism.

2.1.2. OATS

The Older Australian Twins Study (OATS) is a purposeful sample of elderly twins (and some of their siblings) aged 65 years and older, mainly recruited through the Australian Twin Registry (www.twins.org.au), existing studies and advertisement. The sample receives a comprehensive neuropsychological assessment at baseline, with a repeated assessment every two years. The exclusion criteria included: diagnosis of malignancy or other life-threating medical illness, inadequate English to complete the neuropsychological assessment, and current diagnosis of acute psychotic disorder. For detailed information regarding OATS see Sachdev et al., 2009. For the present study one individual of each family with genotyping data available for *KIBRA* and *CLSTN2* and with a MMSE score > 24 at Wave 1 and 2 was included in the analysis, giving a maximum sample size of 279 individuals (179 women) for neuropsychological performance at Wave 1 and 215 individuals at Wave 2 (134 women). Structural MRIs were available from 192 individuals (124 women) at Wave 1 and 137

individuals (87 women) at Wave 2. The time interval between Waves was 2 years. The age of the participants were distributed accordingly: 57.9 % were 65-70 years; 23.4 % were 71-75 years; 11.4 % were 76-80 years; and 7.2 % were 81-90 years. See Table 1 (full sample) and Table 2 (MRI-sample) for demographic data including age, education, and health variables split by the *KIBRA* polymorphism. See supplementary Table S1 (full sample) and S2 (MRI-sample) for demographic information split by the *CLSTN2* polymorphism.

- Table 1 about here –

- - Table 2 about here -

2.2. Neuropsychological testing

An extensive battery of neuropsychological tests was administered, for both Sydney MAS and OATS, targeting different cognitive domains. For the present study measures of episodic memory were of primary interest. The memory tests included story recall (Logical Memory Story A, Wechsler, 1997), Rey Auditory Verbal Learning Test (RAVLT, Strauss, Sherman, & Spreen, 2006), and the Benton Visual Retention Test Recognition test (BVRT, Benton, Sivan, & Spreen, 1996). Domain scores rather than single memory test results were used in this analysis to minimize multiple testing issues. In the present study two different domain scores were used, covering (i) general memory domain and (ii) verbal memory domain. The former included the tests of Logical Memory delayed recall, the RAVLT delayed recall and the BVRT, but the latter included only the two verbal memory tests and excluded the BVRT. Data cleaning was performed and any outliers were either deleted or winsorised according to decisions by clinical experts. To calculate the domain scores for both Sydney MAS and OATS, the raw test scores were z-transformed first transformed to Z-scores using the means and SDs of participants' scores within each study at baseline. The average of these Z-scores

where then calculated, and Domain scores formed by transforming these composites to Z-scores, using the means and SDs of the composites at baseline in each study. The baseline samples used in the two studies for the calculation of the above means and SDs differed slightly, in that the Sydney MAS reference group comprised only those participants in the baseline cohort that were free of medical conditions that could be expected to affect cognitive performance, whilst for OATS the entire OATS baseline sample was used.

2.3. Genotyping

The genotypes for the *KIBRA* rs17070145 and *CLSTN2* rs6439886 SNPs were used in these analyses.

Sydney MAS: DNA samples were genotyped using the Affymetrix Human 6.0 array (California, USA) at the Ramaciotti Centre, UNSW Australia. Genotypes were called in R (v2.12.1), using the CRLMM package (v1.10.0). Briefly, during quality control checks, SNPs were excluded if the minor allele frequency was <0.01, if the genotyping call rate was <95%, or if they failed a Hardy-Weinberg equilibrium threshold of $<1 \times 10^{-6}$. Participants were excluded if the genotyping call rate was <95%. Relatedness and sex checks were undertaken. Any ethnic outliers were excluded. After quality control procedures were completed, there was genetic data on 734,550 SNPs. For further details see Mather et al (2015).

OATS: Genotyping was undertaken using the Illumina Omni Express array (California, USA) at the Queensland Diamantina Institute, Brisbane, Australia. Genotypes were called using the Illumina Genome Studio V2011.1. Quality control checks were similar to the Sydney MAS cohort. After quality control procedures were complete there was genotyping data on 646,791 SNPs. For further details see Mather et al (2015).

The two SNPs were in Hardy-Weinberg equilibrium in both cohorts and the allele frequencies (Table 3) were similar to other Caucasian populations (e.g., Preuschhof et al., 2010). Consistent with previous studies (e.g. Papassotiropoulos et al., 2006; Preuschhof et al., 2010) we combined the two genotype groups carrying the T-allele of the *KIBRA* (T-carriers) as well as the two genotypes carrying the C-allele of the *CLSTN2* (C-carriers). There was no significant difference in background variables in any of the samples between the two different *KIBRA* genotype groups (see Table 1), or between the four different *KIBRA*/*CLSTN2* genotype groups (data not shown). *APOE* genotyping was performed using Taqman genotyping assays for the two SNPs, rs429358 and rs7412 to genotype the three *APOE* alleles, ε2/3/4 as per Song et al. 2012. Participants were classified as an *APOE* ε4 carrier or not.

2.4. Structural MRI

For the Sydney MAS sample, approximately half the participants were scanned using a Philips 3T Intera Quasar scanner (Philips Medical Systems, Netherlands) whereas the remaining participants were scanned on a Philips 3T Achieva Quasar Dual scanner. At the second Wave of data collection all brain scans were undertaken using the Achieva Quasar Dual scanner. The scanning took place at the Prince of Wales Medical Research Institute, Sydney. For all T1-weighted structural images the following parameters were used: TR = 6.39 ms, TE = 2.9 ms, flip angle = 8°, matrix size = 256 x 256, field of view = 256 x 256 x 190 mm^3, slice thickness = 1 mm with no gap between and voxel size 1 x 1 x 1 mm^3. For further details see Jiang et al. (2013). For the OATS sample, MRI scanning took place on 1.5 T scanners with matched and standardized acquisition protocols at 3 imaging centers due to the number of sites used in this study. One center initially used a 1.5 T Philips Gyroscan scanner (Philips Medical Systems, Best, Netherlands) then subsequently a 3T Philips Achieva Quasar Dual scanner. The other two centers used a Siemens Magnetom Avanta and Sonata Scanners

(Siemens Medical Solutions, Malvern PA, USA). In total, four different scanners were used in Wave 1 data collection, and three different scanners for Wave 2 data collection. For structural T1 images the following parameters were used: in-plane resolution = 1x1mm; slice thickness = 1.5mm; number of slices 144; TR = 1530 ms; TE = 3.24 ms; inversion time = 780 ms; flip angle = 8°; number of excitations = 1. For further details see Batouli et al. (2014).

Hippocampal volume was obtained by standard tracing protocol using the FMRIB's software Library v5. 2.0 (www.fmrib.ox.ac.uk/fsl). Firstly non-brain tissue was removed, by warping a brain mask defined in the standard space back to the T1-weighted structural MRI scan. The brain mask was obtained with an automated skull stripping procedure based on the SPM8 skull-cleanup tool (Ashburner, 2009). The FMRIB's Linear Image Registration Tool (FLIRT, v5.5) was then used to linearly register the second brain scan of each participant to the first scan. FMRIB's Integrated Registration and Segmentation Tool (FIRST v4.1) was used to generate hippocampal volumes. Intracranial volume (ICV) was calculated as the sum of grey matter, white matter and cerebrospinal fluid. Visual quality control of the results was performed using ENIGMA protocols (http://enigma.loni.ucla.edu/protocols/imaging-protocols/protocol-for-quality-control-and-summary-statistics/). In short, after running FIRST, registration was checked by extracting three slices each of the coronal, sagittal and axial planes from each linearly transformed brain. For comparison, an outline of the templates was mapped onto the slices. We confirmed that the size of the participant's brain corresponded with that of the template, verified that the lobes were appropriately situated, and confirmed that the orientation of the participant matched the template. Finally, segmentations were checked for each subject in terms of whether the labels cover the full hippocampi using the FSLVIEW toolbox. Scans were excluded if they failed visual quality control (QC). Out of the entire MRI sample for Sydney MAS (N=551), 3 (0.5%) of the left hippocampi and 4 (0.72%)

of the right hippocampi scans failed QC. For OATS (N=414), 6 (1.44%) and 5 (1.21%) for the left and right hippocampi scans respectively were omitted from further analysis.

2.5. Statistical analysis

In all analyses, the first step was to examine main effects of the *KIBRA* polymorphism (T-carriers vs. non-carriers), and the *CLSTN2* SNP. In a second step we investigated the interaction between *KIBRA* and *CLSTN2*. To examine whether the *KIBRA* or *CLSTN2* polymorphisms had any effect on demographic/potentially cofounding variables including sex, *APOE* ε4 status, education, BMI, hip/waist ratio, seated systolic blood pressure (average of two measurements), diastolic blood pressure (average of two measurements) and seated pulse (average of two measurements), χ^2 –tests or ANOVAs were performed. Performance on cognitive variables and hippocampal volume was analyzed using ANCOVAs with *KIBRA* and *CLSTN2* polymorphisms as factors. For cognitive performance, sex, age, education, *APOE* ε4 status, and non-English speaking background were used as covariates. For MRI analyses, sex, age, intra-cranial volume (ICV) and scanner-type were used as covariates. We used *APOE* ε4 status as a covariate in the memory analysis based on previous studies showing that *APOE* ε4 is negatively related to memory performance in older individuals (Albert et al., 2014; Nilsson et al.,2006). We did not include *APOE* ε4 status as a covariate in the hippocampal volume analyses based on studies showing that *APOE* ε4 status is not associated with reduced HC volume in older individuals (Taylor et al., 2014; Mather et al., 2015). To analyze change in cognitive domains and hippocampal volume over time we used repeated measures ANCOVAs again with *KIBRA* T-status and *CLSTN2* as factors. We used the same covariates in the longitudinal analysis as was used in the cross-sectional analysis. We first performed the analyses on the Sydney MAS sample, followed by the OATS sample, and all analyses were done using SPSS version 22. In order to increase statistical power we also evaluated the main

effect of the *KIBRA* and *CLSTN2* SNPs and their interaction on memory performance, memory decline, hippocampal volume and hippocampal atrophy by combining the data from the Sydney MAS and the OATS cohorts. For this analysis we also added study cohort as covariate.

3. Results

3.1. KIBRA and CLSTN2 main effects

3.1.1. Cross-sectional analysis

Table 2 shows memory domain scores and hippocampal volume, including statistics, split by the *KIBRA* SNP T-carrier status for the two cohorts, Sydney MAS and OATS. When comparing memory domain scores at Wave 1 from the Sydney MAS cohort there was no beneficial effect of being a *KIBRA* T-carrier on any of the variables: General memory domain $p = .79$; Verbal memory domain $p = .72$. Nor were there any significant main effects for *CLSTN2* carrier status (general memory domain $p = .62$, verbal memory domain $p = .64$). There was no difference in hippocampal volume between *KIBRA* T-carriers and non-carriers, right HC $p = .78$; left HC $p = .93$, or an effect of *CLSTN2* carrier status, right HC $p = .69$; left HC $p = .64$. Similar results were observed for OATS. See Table 3 for data split by the *KIBRA* polymorphism and supplementary Table S3 for data split by the *CLSTN2* polymorphism.

-Table 3 about here-

3.1.2. Longitudinal analysis

For the Sydney MAS cohort there was a significant decline in performance for the general memory domain $p < .0001$, and verbal memory $p = .001$ over two years. However, as shown

in Table 2, memory decline was not associated with the *KIBRA* polymorphism (memory domain p = .63; verbal memory p = .71), or with the *CLSTN2* polymorphism (memory domain p = .77, verbal memory p = .49). In the Sydney MAS cohort there was a biannual reduction in left hippocampal volume of 3.4 % (p = .9) and 2.7 % in right HC volume (p = .5) between Wave 1 and 2. This is in line with previous studies reporting an annual atrophy rate of 0.8 - 1.55 % (Barnes et al., 2009; Du et al., 2006; Fjell et al., 2009) There was no relationship between the *KIBRA* polymorphism and longitudinal atrophy of either the right (p = .58) or left hippocampus (p = .40) or with the *CLSTN2* polymorphism (right HC p = .74, left HC p = .99). Total HC volume biannual atrophy was 3.0 % for MAS (p = .7) and 2.4 % for OATS (p = .2), which is within the normal annual atrophy rate presented in previous studies (e.g., Barnes et al., 2009). For the OATS cohort there was a 2.4 % reduction in right HC volume (p = .1) and 2.3 % reduction in left HC volume (p = .6) between Waves, with no relationship to *KIBRA* or *CLSTN2* polymorphisms. See Table 3 for data split by the *KIBRA* polymorphisms and supplementary Table S3 for data split by the *CLSTN2* polymorphism.

3.2. KIBRA x CLSTN2 interaction effects

3.2.1. Cross-sectional analysis

See Table 3 for memory domain scores and right and left hippocampal volume split by the four *KIBRA* and *CLSTN2* genotype carrier groups, including statistics. For the Sydney MAS cohort, when examining the *KIBRA* x *CLSTN2* effects, there were no significant interactions on any of the memory measures at Wave 1: General memory domain p = .57; verbal memory domain p = .88. After controlling for sex, age, scanner-type and ICV there was a significant interaction between the genotype groups for the right hippocampal volume (p = .02), but here was no such effect on the left hippocampus (p = .10). Post hoc testing of the right HC volume revealed no further differences between the four genotype groups. Moreover, the results for

the right hippocampus would not survive correction for multiple testing. Thus, we are unable to show any genetic effects on hippocampal volume by the *KIBRA* or *CLSTN2* polymorphisms for Sydney MAS. The OATS did not find any significant KIBRA x CLSTN2 interaction effects on memory performance nor hippocampal volume.

3.2.2. Longitudinal analysis

For the Sydney MAS cohort, longitudinal analyses of change in cognitive performance and hippocampal volume did not show signs of an interactive effect of *KIBRA* x *CLSTN2* polymorphisms on neither decline in performance on the memory measures: general memory domain p = .33; verbal memory domain p = .57, nor on the change of hippocampal volume (right HC p = .11; left HC p = .64). See Table 4 for memory scores and hippocampal volume split by *KIBRA* and *CLSTN2* polymorphism carrier status, including statistics. Similar results were observed in the OATS cohort.

-Table 4 about here -

3.3. Combining Sydney MAS and OATS cohorts

The results when combining the two cohorts did not reveal any significant main effects of either *KIBRA* (general memory domain p = .67; verbal memory domain p = .89; left HC p = .70; right HC p = .79) or *CLSTN2* (general memory domain p = .14; verbal memory domain p = .058; left HC p = .86; right HC p = .36) SNPs on memory performance or hippocampal volume. There were no interactive effects of these two polymorphisms (general memory domain p = .97; verbal memory domain p = .81; left HC p = .27; right HC p = .13). Similar results were found for the longitudinal analysis of memory decline and hippocampus atrophy: *KIBRA* (general memory domain p = .26; verbal memory domain p = .93; left HC p = .68;

right HC p = .42); *CLSTN2* (general memory domain p = .64; verbal memory domain p = .39; left HC p = .62; right HC p = .76); *KIBRA* x *CLSTN2* (general memory domain p = .86; verbal memory domain p = .95; left HC p = .39; right HC p = .17).

3.3. Supplementary material

In previous genetic studies total hippocampal volume has been assessed rather than left and right hippocampal volumes (Palombo et al., 2013), and measures of immediate recall of episodic memory tests have shown the strongest effect of the *KIBRA* polymorphism (Kauppi et al., 2011). Therefore we also investigated the potential effects of the *KIBRA* and *CLSTN2* SNPs on total hippocampal volume (left + right) and on performance of immediate recall test specifically. The results confirmed our previous findings showing no association with *KIBRA* or *KIBRA*/*CLSTN2* in both Sydney MAS and OATS (see Supplementary Table S4 and S5).

4. Discussion

In the present study, by using both cross-sectional and longitudinal data, we investigated if genetic polymorphisms in *KIBRA* and *CLSTN2* were associated with memory performance and hippocampal volume in two independent samples of older adults. This is the first study to also examine change of hippocampal volume in relation to *KIBRA* and *CLSTN2* polymorphisms longitudinally. We failed to replicate the initial reported association of the *KIBRA* T-allele with better long-term memory performance (Papassotiropoulos et al., 2006) as well as the suggested favorable interactive effect of *KIBRA* T-carriers and *CLSTN2* C-carriers (Preuschhof et al., 2010). Moreover, in the present samples, hippocampal volume was not associated with the *KIBRA* SNP alone or in combination with the *CLSTN2* SNP, and the results did not support the influence of the *KIBRA* SNP, with or without the *CLSTN2* SNP, on longitudinal memory decline or hippocampal atrophy in older adults.

A number of studies have examined the role of the *KIBRA* polymorphism on memory performance. The results are, however, inconsistent with studies showing a beneficial effect of the T-allele (Papassotiropoulos et al., 2006; Kauppi et al., 2011), or no association (Need et al., 2008) and even negative effects (Nacmias et al., 2008). In our samples there was no *KIBRA* T-allele effect, alone or in combination with *CLSTN2* C-allele, on memory performance. Our results are in line with the Sedille-Mostafaie et al. (2012) study which investigated the main effect of *KIBRA* and *CLSTN2* polymorphisms in an older sample (age range 75-76 years), and stand in contrast to the study by Preuschhof et al. (2010) who analyzed the interactive effect between the two SNPs in a younger sample (age range 20-31). Hence, it is possible that the effect of *KIBRA* and *CLSTN2* may be diluted in older samples potentially because of the influence of age-related neuropathological changes on memory performance, which may interact with polymorphisms such as *KIBRA* and *CLSTN2*. Some additional support for this suggestion comes from a cross-sectional study of older individuals (> 60 years) with or without depression in which Pantzar et al. (2014) showed that it was only an interactive effect of *KIBRA* and *CLSTN2* polymorphisms on memory performance in individuals with unipolar depression, but not in older individuals without depression. However, it should be noted that based on the available literature it is difficult to conclude that there indeed is a *KIBRA*/*CLSTN2* age-effect since studies using samples with an age over 50 years have been both in favor (e.g., Schaper et al., 2008) and against (Nacmias et al., 2008; Rodríguez-Rodríguez et al., 2009) the positive influence of the *KIBRA* T-allele on memory. Less work has examined the *CLSTN2* SNP and memory across the lifespan. Notably, in one study examining the effect of the *KIBRA* T-allele in a spatial navigation task, the reversed was observed. Older (60-70 years) T-allele carriers performed better than older CC-carriers but no such pattern was observed for younger (19-30 years) individuals (Schuck et al., 2013). Future

studies should undertake a more detailed examination of the age-interaction with *KIBRA* or *CLSTN2* status to clarify these relationships.

Recent findings from the OATS cohort suggest that even though the genetic impact on regional brain volumes is reduced with age, it is still relatively high (63% for total brain volume) late in life (Batouli, Trollor, Wen, & Sachdev, 2014). Importantly, hippocampal volume also has high heritability in older adults (~62%) (Mather et al., 2015). In our older samples, we did not find support for a *KIBRA* SNP effect on hippocampal volume, indicating that other genes than *KIBRA* may be responsible for the genetic impact on hippocampus volume. This is consistent with Papassotiropoulos et al., 2006, but in contrast to the recent findings of Palombo et al., (2013), who found that the T-allele was associated with greater hippocampal volume using a small cohort of younger (mean age 22 years) adults (N=32). In combination with the *CLSTN2* SNP there was a small interactive effect in the Sydney MAS sample for right hippocampal volume but it did not survive correction for multiple testing. Further post-hoc testing could not separate between the four different genotype groups. Moreover, this effect was not replicated in the smaller OATS sample, or when combining the two cohorts. Thus, we do not find evidence that *KIBRA* or *CLSTN2* polymorphisms influence memory performance or hippocampal volume in our elderly cohorts.

Limitations of this study include the relatively short follow-up time interval of two years between Waves of data collection, however the hippocampal atrophy observed was comparable to previous reports in normal aging (e.g., Barnes et al., 2009). As our studies continue to collect data, it will facilitate a more detailed examination of the influence of genetic variation in *KIBRA* and *CLSTN2* on longitudinal memory performance and change in hippocampal volume. Also, even when we combined our cohorts to increase statistical power our sample size was relatively small, and a recent study indicated that in order to have 80 % power to detect an effect of *KIBRA* on episodic memory it requires a sample size of ~2000

individuals (Milnik et al., 2012). However, our subsample with hippocampal volume data was comparatively larger than most other studies previously reported (e.g. Papassotiropoulos et al., 2006; Palombo et al., 2013). In addition, we explored the relationships exclusively in older adults and did not examine age-specific effects across the lifespan. Another methodological limitation of the present study is the large number of different scanners and of different field strengths used during data collection. Even though scanner-type was added as covariate in the analysis, it is possible that this methodological factor contributed to the observed null-findings. However, this is a common problem encountered in similar studies (e.g. Hibar et al., 2015). There are also limitations with the use of automated volumetric estimations of hippocampal volume compared with manual tracing (see e.g., Kennedy et al., 2009) which may also have affected the results. Further, even though we excluded individuals with a low MMSE score, some individuals may have had early-stage dementia or mild cognitive impairment (MCI), which may have influenced our results. For example, Almeida et al (2008) found no relationship between the *KIBRA* SNP and MCI. A final limitation is that when we combined the two samples, the different cohorts used different normative samples when calculating standardized memory composite scores. It is important to stress that even though we did not find any significant association with either *KIBRA* or *CLSTN2* polymorphisms and memory or hippocampal volume, such relationships may still exist, perhaps by including other potential moderators. For example, one study showed that *KIBRA* interacted with gender and arterial hypertension in relation to cognitive performance (Wersching et al., 2011). In the present sample we did not observe any difference in blood pressure between the different *KIBRA* genotype groups and we therefore did not further examine this potential interaction (data not shown).

In summary, considering previous observations, together with those of the present study, the influence of *KIBRA* or *CLSTN2* SNPs on age-related memory performance

is still questionable (Payton, 2009) and difficult to interpret (Milnik et al., 2012; Schneider et al., 2010). As the first longitudinal study to investigate the effects of these SNPs on memory and hippocampal volume we did not find support that these polymorphisms influence age-related memory decline or hippocampal atrophy over two years in our cohorts of older adults. Future studies should investigate longitudinal change over longer time intervals, employ larger samples and further investigate the influence of age on these relationships.

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Table 1. Demographic information for the Sydney Memory and Ageing Study (Sydney MAS) and the Older Australian Twins Study (OATS) for the *KIBRA* rs17070145 T-carrier versus CC genotype groups

	Sydney MAS			OATS		
	KIBRA CC N = 404	*KIBRA* T-carrier N = 508	Statistic, p-value	*KIBRA* CC N = 119	*KIBRA* T-carrier N = 160	Statistic, p-value
Age years (range)	78.8 (70.3-90.0)	78.7 (70.3-90.8)	$F_{(1, 911)}$ =.17, p = .67	71.2 (65-90)	70.4 (65-88)	$F_{(1, 277)}$ =1.5, p = .22
Sex (females)	233 (57%)	275 (54%)	χ^2 (1) = .80, p = .44	72 (61%)	107 (67%)	χ^2 (1) = 1.2, p = .27
Education years (range)	11.7 (5-24)	11.5 (3-23)	$F_{(1, 911)}$ = 1.26, p = .25	11.4 (2-21)	11.2 (6-21)	$F_{(1, 277)}$ =.095, p = .77
APOE ε4	99 (24 %)	113 (22 %)	χ^2 (1) = .53, p = .52	22 (21%)	44 (30%)	χ^2 (1) = 2.3, p = .10
BMI	27.1 (4.3)	27,1 (4.6)	$F_{(1, 908)}$ = .02, p = . 86	27.2 (4.5)	27.4 (4.1)	$F_{(1, 240)}$ =.13, p = .73
Hip/waist ratio	.90 (.08)	.91 (.08)	$F_{(1, 889)}$ = 1.37, p = .16	.89 (.09)	.88 (.09)	$F_{(1, 270)}$ =.44, p = .48
Systolic bp seated	143.9 (21.2)	145.6 (21.6)	$F_{(1, 904)}$ = 1.52, p = .18	139.3 (18.3)	139.4 (20.9)	$F_{(1, 267)}$ =.001, p = .88
Diastolic bp seated	82.0 (12.0)	81.6 (11.0)	$F_{(1, 904)}$ = .29, p = .60	80.4 (10.9)	81.7 (11.6)	$F_{(1, 267)}$ =.04, p = .83
Pulse seated	70.3 (11.9)	68.8 (11.8)	$F_{(1, 901)}$ = 3.53, p = .06	73.4 (12.9)	73.6 (11.5)	$F_{(1, 264)}$ =.1.5, p = .21

Note: Means (standard deviations) or sample size (%) are presented unless otherwise specified

Table 2. Demographic information for the participants with MRI and genetic data of the Sydney Memory and Ageing Study (Sydney MAS) and the Older Australian Twins Study (OATS) split by *KIBRA* rs17070145 T-carrier versus CC genotype groups

	Sydney MAS MRI Sample			OATS MRI Sample		
	KIBRA CC N = 218	*KIBRA* T-carrier N = 283	Statistic, p-value	*KIBRA* CC N = 77	*KIBRA* T-carrier N = 114	Statistic, p-value
Age years (range)	78.7 (70.5-89.1)	78.2 (70.7-90.4)	$F_{(1, 499)}$ =1.1, p = .29	70.5 (65-89)	70.0 (65-84)	$F_{(1, 189)}$ =.43, p = .51
Sex (females)	121 (56%)	157 (56%)	χ^2 (1) = .01, p = .9	45 (58%)	78 (68%)	χ^2 (1) = 2.0, p = .16
Education years (range)	11.8 (5-24)	11.6 (4-23)	$F_{(1, 499)}$ = .40, p = .53	11.4 (6-21)	11.2 (6-21)	$F_{(1, 189)}$ =.20, p = .66
APOE ε4	49 (23 %)	65 (23 %)	χ^2 (1) = .02, p = .9	16 (21%)	32 (28%)	χ^2 (1) = 1.2, p = .27
BMI	26.9 (3.9)	26.7 (4.2)	$F_{(1, 493)}$ = .21, p = .65	27.1 (3.8)	27.5 (4.2)	$F_{(1, 168)}$ =.48, p = .49
Hip/waist ratio	.90 (.08)	.90 (.08)	$F_{(1, 491)}$ = .09, p = .77	.89 (.09)	.88 (.09)	$F_{(1, 186)}$ =.84, p = .36
Systolic bp seated	142.8 (20.3)	146.1 (20.6)	$F_{(1, 486)}$ = 3.1, p = .08	139.4 (17.7)	138.8 (21.1)	$F_{(1, 182)}$ =.04 p = .85
Diastolic bp seated	81.3 (11.0)	82.1 (10.8)	$F_{(1, 486)}$ = .61, p = .44	80.7 (10.9)	80.7 (11.1)	$F_{(1, 182)}$ =.00, p = .98
Pulse seated	69.4 (12.3)	68.2 (11.5)	$F_{(1, 485)}$ = 1.2, p = .27	74.4 (11.7)	72.9 (11.3)	$F_{(1, 181)}$ =.70, p = .40

Note: Means (standard deviations) or sample size (%) are presented unless otherwise specified

Table 3. Memory performance (z-scores) and hippocampal volume (mm^3) at Waves 1 and 2 split by the *KIBRA* SNP rs17070145 (T carriers vs CC) with cross-sectional and longitudinal analysis results presented for both the Sydney MAS and the OATS

	KIBRA CC		*KIBRA* T-carriers		Statistics[a]	
Sydney MAS	Wave 1	Wave 2	Wave 1	Wave 2	Cross-sectional (Wave 1)	Longitudinal
General memory	-.08 (1.0)	-.15 (1.1)	-.11 (1.0)	-.21 (1.1)	$F_{(1, 911)} = .07$, $p = .79$	$F_{(1, 786)} = .23$, $p = .63$
Verbal memory	-.06 (1.0)	-.18 (1.1)	-.10 (1.0)	-.23 (1.1)	$F_{(1, 908)} = .13$, $p = .72$	$F_{(1, 778)} = .14$, $p = .71$
L HC volume	3312.4 (444.2)	3212.0 (445.8)	3315.5 (477.6)	3193.2 (475.6)	$F_{(1, 496)} = .01$, $p = .93$	$F_{(1, 323)} = .70$, $p = .40$
R HC volume	3423.8 (426.0)	3348.5 (487.4)	3440.6 (476.1)	3333.8 (508.0)	$F_{(1, 495)} = .08$, $p = .78$	$F_{(1, 322)} = .58$, $p = .58$
OATS	Wave 1	Wave 2	Wave 1	Wave 2		
General memory	.08 (1.0)	.01 (.8)	.05 (.9)	.16 (.6)	$F_{(1, 233)} = .45$, $p = .52$	$F_{(1, 170)} = 3.1$, $p = .08$
Verbal memory	.07 (1.0)	.02 (.9)	.1 (.8)	.15 (.7)	$F_{(1, 246)} = .0$, $p = .91$	$F_{(1, 178)} = .9$, $p = .33$
L HC volume	3505.7 (443.1)	3382.3 (449.8)	3533.2 (458.9)	3495.3 (478.3)	$F_{(1, 186)} = .70$, $p = .37$	$F_{(1, 117)} = .7$, $p = .38$
R HC volume	3628.5 (447.6)	3527.3 (443.8)	3620.1 (437.5)	3552.3 (439.5)	$F_{(1, 186)} = .0$, $p = .89$	$F_{(1, 117)} = .7$, $p = .42$

Notes. Means (standard deviations) are presented; [a] Results from ANCOVA analyses presented. Memory analyses were adjusted for sex, age, education, *APOE* ε4 status, and non-English speaking background, hippocampal volume analyses were adjusted for sex, age, intra-cranial volume (ICV) and scanner-type.

Table 4. Memory domain performance, z-scores and hippocampal volume (mm^3) at Waves 1 and 2 split by *KIBRA* rs17070145 (T-carriers vs. CC) and *CLSTN2* rs6439886 (C carriers vs. TT) with cross-sectional and longitudinal analysis results presented for both the Sydney MAS and the OATS.

KIBRA	CC		CC		T carriers		T carriers					
CLSTN2	C carriers		TT		TT		C carriers		Statistics[a]			
Sydney MAS	Wave 1 (10.8 %)	Wave 2	Wave 1 (33.7 %)	Wave 2	Wave 1 (40.4 %)	Wave 2	Wave 1 (15.0 %)	Wave 2	Cross-sectional (Wave 1)	p	Longitudinal	p
General memory	-.04 (1.1)	-.14 (1.1)	-.09 (1.0)	-.16 (1.1)	-.10 (1.0)	-.22 (1.0)	-.14 (1.1)	-.20 (1.2)	$F_{(3, 910)} = .21$	.57	$F_{(3, 784)} = .65$	.33
Verbal memory	-.05 (1.1)	-.19 (1.1)	-.06 (1.0)	-.18 (1.1)	-.18 (1.2)	-.18 (1.1)	-.11 (1.1)	-.20 (1.2)	$F_{(3, 906)} = .1$	.88	$F_{(3, 776)} = .61$	.57
R HC volume	3491.6 (428.9)	3442.6 (444.4)	3399.2 (423.6)	3316.3 (498.8)	3467.6 (462.5)	3348.7 (501.0)	3375.8 (462.5)	3292.6 (529.0)	$F_{(3, 493)} = 2.8$	.02	$F_{(3, 321)} = 2.0$	.11
L HC volume	3397.3	3298.4	3281.6	3181.8	3328.8	3211.8	3283.9	3140.9	$F_{(3, 494)} = 2.1$	.10	$F_{(3, 319)} = .56$	.64

	(510.5)	(453.0)	(415.0)	(441.1)	(476.4)	(482.1)	(481.7)	(456.9)				
OATS	Wave 1 (11.5 %)	Wave 2	Wave 1 (31.2 %)	Wave 2	Wave 1 (44.8 %)	Wave 2	Wave 1 (12.5 %)	Wave 2				
General memory	.04 (1.0)	-.10 (1.0)	.09 (1.0)	.04 (.7)	.01 (.8)	.12 (.6)	.18 (.9)	.25 (.6)	$F_{(3, 231)} = .71$	.53	$F_{(3, 168)} = 1.2$	.28
Verbal memory	-.07 (1.1)	-.15 (1.1)	.11 (.9)	.06 (.9)	.10 (.9)	.14 (.7)	.13 (.8)	.20 (.7)	$F_{(3, 244)} = .51$	.71	$F_{(3, 176)} = .33$	.76
R HC volume	3610.5	3485.0	3635.2	3538.8	3627.8	3584.0	3597.5	3450.1	$F_{(3, 184)} = .13$	.94	$F_{(3, 115)} = .7$	.64
	(423.9)	(483.6)	(459.8)	(437.2)	(441.1)	(451.2)	(433.7)	(393.7)				
L HC volume	3478.0	3301.8	3516.5	3404.1	3522.3	3498.6	3566.3	3484.6	$F_{(3, 184)} = .7$	.5	$F_{(3, 115)} = .6$	.62
	(485.0)	(374.1)	(429.7)	(469.3)	(452.3)	(499.4)	(485.5)	(415.7)				

Notes. Means (standard deviations) are presented; [a] Results from ANCOVA analyses presented. Memory analyses were adjusted for sex, age, education, *APOE* ε4 status, and non-English speaking background, hippocampal volume analyses were adjusted for sex, age, intra-cranial volume (ICV) and scanner-type.

Highlights

Two independent cohorts were used to study the cross-sectional and longitudinal influences of *KIBRA* and *CLSTN2* SNPs on memory and hippocampal volume

No main or interactive effect of *KIBRA* SNP or *CLSTN2* SNP on memory or hippocampal volume

No main or interactive effect of *KIBRA* or *CLSTN2* SNPs on hippocampal atrophy or memory decline