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KIBRA genetic polymorphism influences episodic memory in later life, but does not increase the risk of mild cognitive impairment

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Abstract

A common T→C polymorphism of the KIBRA gene has been recently associated with worse performance on tests of episodic memory. This should aimed to determine whether older adults with the KIBRA CC genotype (1) have worse episodic memory than T-allele carriers and, (2) are more likely to express the phenotype of amnestic mild cognitive impairment (MCI). Our Cross-sectional investigation of 312 adults aged 50–89 years free of dementia included genotyping of the KIBRA rs17070145 gene and the assessment of episodic memory to Establish a Registry for Alzheimer's Disease (CERAD). Participants were considered to have MCI if their memory scores were 1.5 standard deviations below the mean norm for the population. 138/312 participants carried the KIBRA CC genotype. Their immediate and delayed recall scores were significantly lower than the scores of carriers of the T allele ($P < 0.05$; adjusted for age, gender and premorbid IQ), although the effect size of the CC genotype was weak (0.2). Amongst our volunteers, 133 had MCI, of whom 63 (47.4%) had the CC genotype. There was no association between KIBRA genotype and MCI phenotype (TT/CT *versus* CC; adjusted odds ratio = 1.70, 95%CI = 0.74, 3.90). We concluded that the KIBRA T→C polymorphism contributes to modulate episodic memory amongst community-dwelling older adults free of dementia, but plays no obvious role in the phenotypic expression of MCI. Future studies should aim to clarify the long term implications of this polymorphism on cognitive function and to identify other genes involved in the modulation of memory that might confer greater risk of MCI in later life.

Keywords: KIBRA • mild cognitive impairment • Alzheimer's disease • genetics • memory • episodic memory • cognitive performance • ageing • elderly

Introduction

Memory is a fundamental cognitive ability that enables humans to learn and remember information, contributing to fill people's lives with meaning, continuity and context. Memory consists of procedural and declarative systems, with the latter including semantic and episodic memory [1]. Episodic memory represents a set of cognitive functions involved in the encoding, storage and retrieval of information about facts or events that happen within a particular time and place. There is now evidence that episodic memory is a polygenic cognitive trait with estimated heritability of 50% in twin studies [2]. Recently, Papassotiropoulos and colleagues [3] showed that a single nucleotide polymorphism (T→C) within the ninth intron of the

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KIBRA gene (rs17070145) influences episodic memory and modulates the activation of the hippocampus during memory retrieval. They studied three independent samples of adults and found that those with the CC genotype had significantly worse scores on tests of verbal and visual episodic memory than CT and TT carriers. The same pattern of memory performance according to KIBRA polymorphisms was observed in 64 healthy older adults from Germany [4], but data about the relevance of this polymorphism in later life remains sparse and it is unclear whether this T→C substitution contributes to modulate episodic memory in older people.

The performance of adults in tests of episodic memory declines with increasing age.5 In some, these deficits do not seem to be progressive in nature, but in others they are the precursors of further cognitive decline and dementia [6, 7]. The concept of 'mild cognitive impairment' (MCI) was introduced to describe a group of people who are at increased risk of developing dementia as they age [7]. Typically, people with MCI show normal age-adjusted general cognitive abilities, but display significant deficits in tests of episodic memory [7]. Progress to dementia ranges from 5 to 16% per annum [8], but epidemiological evidence indicates that most people with MCI do not progress to a full-blown dementia syndrome 2 years after diagnosis [9]. These results suggest that the genetic determinants of MCI and dementia phenotypes are not necessarily the same, and raise the possibility that the KIBRA T→C polymorphism has a role to play in the development of MCI in later life.

We designed this study to: (1) replicate the findings of Papassotiropoulos *et al.* in an independent sample of community-dwelling older adults and, (2) determine if the MCI phenotype is associated with T→C polymorphism of the KIBRA gene.

Methods

Participants

Three hundred and thirty-six adults volunteered to take part in this study. They were recruited through advertisement in the local media and from the local Memory Clinic, and were included if they were aged 50 years or older, had Mini-Mental State Examination (MMSE) score ≥24 [10] and Clinical Dementia Rating (CDR) ≤ 0.5 [11],and showed no evidence of dementia according to the DSM-IV diagnostic criteria (APA, 1994) [12]. We excluded people with self-reported prior clinical history of strokes, severe visual or hearing impairment, a score greater than 18 on the Beck Depression Inventory [13], and those who did not provide written informed consent. The Ethics Committee of the Royal Perth Hospital approved this research project.

Assessment procedure

Three hundred and twelve people were deemed suitable for inclusion in the study (DNA samples were not available for 2 subjects and another 22 volunteers were excluded because they had a MMSE<24 or met criteria for the diagnosis of dementia). Their mean age was 71.2 years (SD = 7.6; range = 50–89) and 50.0% were male. Information on ethnicity was not collected in a structured manner, but we estimated that 98% of participants were of Caucasian background. Participants underwent assessment with the Cambridge Contextual Reading Test (CCRT), which produces a valid measure of the pre-morbid intelligence quotient (IQ) [14] and the cognitive battery of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) [15]. The CERAD is a neuropsychological battery that consists of tests assessing verbal fluency, naming, praxis, immediate and delayed recall (episodic memory), and recognition. Normative data is available for each one of the tests [15]. For the purposes of this study, we grouped participants under the heading of MCI if they had an immediate or delayed recall score on the CERAD that was 1.5 standard deviations below the norm and offered an affirmative answer to the question: 'Do you have any difficulty with your memory?'

Genotyping of the KIBRA gene

Participants donated a blood sample for extraction of DNA and genotyping of the KIBRA SNP rs17070145 using restriction polymerase chain reaction-fragment length polymorphism (PCR-RFLP) analysis. The relevant primers were designed using the web-based Primer3 software [16]: *17070145F2- tttactcccagcacacacctc and 17070145Rev2 - cacaatgaacaaggctgtgg*. The PCR reactions contained 12.5ng genomic DNA, 2pmol each primer, 100uM each dNTP, 1X *Taq polymerase* buffer and 0.5U *Taq Polymerase* (Qiagen) in a final volume of 10ul. Cycling conditions comprised an initial denaturation of 95°C for 5 min., followed by 35 cycles of denaturation at 94°C for 30 sec., annealing at 59°C for 30 sec., extension at 72°C for 30 sec., and a final elongation at 72°C for 7 min. Restriction enzyme digestion of the 160bp PCR product was performed with 2.5U of the restriction enzyme MnlI (New England Biolabs) according to manufacturer's directions. The digested products were separated by 2.5% agarose gel electrophoresis and visualized with ethidium bromide staining. A fragment size of 122bp, represented the T allele (28 and 10bp fragments not visible) and 68bp and 54bp the C allele (28 and 10bp fragments not visible).

Test for Hardy-Weinberg equilibrium showed that the genotypic distribution of rs17070145 did not deviate from expectation (exact probability, $P = 0.524$).

Analysis of data

Data were analysed with the statistical package Stata release 9.2 (StataCorp, College Station, Texas). We used graphic methods to investigate the distribution of the data and statistic summary measures to describe study variables, including proportions, means and corresponding 95% confidence interval (95%CI), and medians with corresponding inter-quartile ranges (IQR). We used Pearson chi-square statistic ($\times^2$) to evaluate the distribution of categorical variables (such as gender) according to the KIBRA polymorphism, Student's t-test to compare means and Mann-Whitney test z-statistic to compare the distribution of ranked data according to KIBRA genotype. Analyses of co-variance were used to determine between-group differences on measures of episodic memory taking into account age and pre-morbid IQ. We used logistic regression to ascertain the risk (odds ratio) of MCI according to KIBRA

Table 1 Clinical and demographic characteristics of older adults according to the KIBRA rs17070145 polymorphism

	TT/CT (n=174)	CC (n=138)	Statistic	*P*
Age, mean years (95%CI)	72.2 (71.1, 73.4)	69.9 (68.7, 71.2)	t = 2.68	0.008
Female gender, n (%)	90 (51.7)	66 (47.8)	chi-square = 0.47	0.494
IQ, mean (95%CI)	115.5 (114.6, 116.4)	116.5 (115.5, 117.4)	t = -1.44	0.152
MMSE, median (IQR)	28 (28, 28)	28 (28, 29)	z = 0.20	0.839
CERAD immediate recall, mean (95%CI)	18.6 (18.0, 19.3)	18.2 (17.4, 18.9)	F = 4.08	0.044*
CERAD delayed recall, mean (95%CI)	5.9 (5.5, 6.2)	5.7 (5.3, 6.1)	F = 3.91	0.049*
CERAD recognition, mean (95%CI)	19.2 (18.9, 19.4)	18.9 (18.6, 19.4)	F = 5.36	0.021*

95%CI: 95% confidence interval, IQ, pre-morbid intelligence quotient; MMSE, mini-mental state examination; IQR, inter-quartile range.
The number of degrees of freedom was 310 for t-tests and 1 for Pearson's chi-square and F statistic (analysis of co-variance)
* *P*-value adjusted for the effect of age, gender and pre-morbid IQ

rs17070145 genotype, taking into account the effects of age, gender and pre-morbid IQ. The Breusch-Pagan / Cook–Weisberg test for heteroskedasticity showed that our data met the assumptions of homoskedasticity. Residuals from the regression models were normally distributed (Shapiro–Wilk $P > 0.28$ for all tests). α was set at 5% and all *P*-values reported are two-tailed.

Results

There were 138, 143 and 31 CC, CT and TT KIBRA rs17070145 carriers in the sample. Table 1 summarizes their clinical and neuropsychological characteristics. People with CC genotype were younger than CT/TT participants (P=0.008). There was also a non-significant trend for CC carriers to have higher pre-morbid IQ (P=0.152). Subjects with the CC genotype of the KIBRA gene had lower scores than their counterparts on immediate and delayed recall, as well as recognition (Table 1). The effect size of KIBRA CC genotype on delayed memory scores was 0.2 (adjusted for age, gender and pre-morbid IQ).

One hundred and thirty-six people met the study criteria for the diagnosis of amnestic MCI, of whom 65 (47.8%) were CC carriers. The genotypic distribution of the KIBRA gene in this subsample was in equilibrium (Hardy-Weinberg test, exact probability, P=1.000). The KIBRA CC genotype was not associated with a significant increase in the odds of MCI (OR = 1.73, 95% CI = 0.75, 3.96, P=0.197; adjusted for age, pre-morbid IQ and gender). We then assessed the effect of the CC genotype on the memory scores of subjects with MCI. The results of these analyses appear in Table 2. There were no obvious associations between KIBRA genotype and memory scores. No further analyses were carried out because of increased risk of type I error and decreased power.

Discussion

We designed this study to address two questions about the role of the KIBRA T→C polymorphism on episodic memory. Firstly, we wished to replicate the original findings of Papassotiropoulos and colleagues [3] in a new sample of community-dwelling older adults without dementia. Our results confirmed that people with the CC genotype have worse episodic memory than carriers of the T allele. Secondly, we wanted to clarify whether the KIBRA gene contributed to the expression of the MCI phenotype in adults aged 50 years or over. We found an excess risk of MCI amongst those with the CC genotype (73%), although this association was not significant (95%CI of the odds ratio = 0.75, 3.96).

It is unclear how well our volunteers represent the population of adults older than 50 years living in the Australian community, but an over-representation of people with memory problems is likely due to the strategies used to recruit participants. Our study sample had a similar proportion of men and women, which is in contrast with census data showing an excess of women in this age group [17]. In addition, the pre-morbid intelligence quotient of participants was above average. As IQ and memory scores have a correlation of approximately 0.5 [18] one might expect a lower prevalence of factors that compromise memory performance among people with above average IQ, such as the KIBRA CC genotype. The prevalence of this genotype in our sample was 44.2%, which is not dissimilar from the 47.5% and 45.3% point prevalence reported by others

Table 2 Clinical and demographic characteristics of older adults with MCI according to the KIBRA rs17070145 polymorphism

	TT/CT (n=71)	CC (n=65)	Statistic	*p*
Age, mean years (95%CI)	72.1 (70.5, 73.7)	69.7 (67.7, 71.6)	t = 1.93	0.055
Female gender, n (%)	43 (60.6)	28 (43.1)	chi-square = 3.22	0.073
IQ, mean (95%CI)	113.8 (112.4, 115.1)	114.8 (113.6, 116.0)	T = -1.13	0.261
MMSE, median (IQR)	28 (27, 28)	27 (27, 28)	Z = -0.02	0.984
CERAD immediate recall, mean (95%CI)	15.2 (14.3, 16.0)	15.2 (14.4, 16.0)	F = 0.11	0.732*
CERAD delayed recall, mean (95%CI)	4.2 (3.6, 4.7)	4.1 (3.6, 4.6)	F = 1.41	0.237*
CERAD recognition, mean (95%CI)	18.5 (18.1, 18.9)	18.2 (17.7, 18.7)	F = 1.69	0.196*

95%CI: 95% confidence interval, IQ, premorbid intelligence quotient; MMSE, mini-mental state examination; IQR: inter-quartile range.
The number of degrees of freedom was 310 for t-tests and 1 for Pearson's chi-square and F statistic (analysis of co-variance)
* *P*-value adjusted for the effect of age, gender and premorbid IQ.

[3, 4]. Moreover, test for Hardy-Weinberg equilibrium revealed no deviation from the expected genotypic distribution, indicating that population stratification is unlikely. Finally, the higher IQ of our participants might have contributed to mitigate the association between KIBRA CC genotype and memory function. We found that the adjusted effect size of this association in our sample for delayed recall was 0.2 (unadjusted effect size of 0.1). Papassotiropoulos and colleagues observed a negative unadjusted effect size of the CC genotype of approximately 0.7 in their first Swiss sample, and 0.4 for both the American and the second Swiss samples [3].

Another factor to be considered in the interpretation of our results relates to the nature of the episodic memory task used in this and other studies. The episodic memory test of the CERAD consists of a list of 10 semantically unrelated words that subjects are asked to read aloud and remember. The immediate recall score is calculated by adding the total number of words remembered correctly immediately after each of the three presentation trials (possible score range: 0–30). Of note, the approach used to calculate CERAD immediate recall scores involves a learning component that is likely to be hippocampus dependent. After a delay of approximately 5 min., subjects are encouraged to remember as many words as they can (possible score range: 0–10) and, later, have to recognize the 10 familiar words in a list of 20 words (possible score range: 0–20). This test is similar to those used by Papassotiropoulos and colleagues [3], although their assessments were more demanding in terms of the number of items to be recalled (15–30), time between presentation and recall (5 min. to 24 hrs), and type of stimulus used (verbal and visual). Hence, our assessment might have been less sensitive.

Papassotiropoulos and co-workers also found that, compared to carriers of the T allele, normal adults with the KIBRA CC genotype displayed greater functional MRI activation of medial temporal and frontal lobe structures, as well as inferior parietal lobule during retrieval of episodic memory information [3]. They concluded that non-carriers of the T allele required more widespread activation (effort) of memory networks to complete the task. Similar findings have been reported for people with MCI [19] albeit not consistently [20].

Of interest, Rodriguez-Rodriguez and colleagues [21] found, in a cross-sectional study of 391 patients with Alzheimer's disease (AD) and 428 healthy controls, that carriers of the KIBRA T allele were more frequent in the AD group. They argued that their findings were in contrast with those of Papassotiopoulos *et al.* [3], although the results could be explained by type I error due to multiple comparisons and survivorship bias leading to the premature censoring of people with AD and the KIBRA CC genotype.

This study has the merit of being the first to analyse the impact of the common KIBRA T→C polymorphism on the phenotypic expression of amnestic MCI. Typically, people with MCI perform poorly on tests of episodic memory, but only a relative small proportion progress to a full blown dementia syndrome at follow-up [9]. This observation suggests that genetic factors other than those related to dementia modulate the expression of the MCI phenotype later in life, although our results indicate that the KIBRA gene is unlikely to play a prominent role in this process. We did not find evidence in support of our original hypothesis that CC carriers would be at greater risk of MCI (although we did observe a trend in that direction), nor were we able to show any evidence of an effect of the gene on the memory scores of older people with MCI. These results suggest that the effect size of the T→C polymorphism decreases with increasing impairment of episodic memory, and that the KIBRA gene plays all but a limited role after scores fall below a certain threshold, as is the case in MCI.

In summary, our results confirm that older adults with the KIBRA CC genotype have slightly worse episodic memory than T-allele carriers. We also found that the KIBRA gene does not play a significant role in the phenotypic expression of amnestic MCI. Future studies should aim to clarify the long term implications of the KIBRA T(C polymorphism on cognitive function and to identify other genes involved in the modulation of memory that might confer greater risk of MCI in later life.

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