

Effects of the KIBRA Single Nucleotide Polymorphism on Synaptic Plasticity and Memory: A Review of the Literature

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Abstract: There has been a great deal of interest recently in genetic effects on neurocognitive performance in the healthy population. KIBRA –a postsynaptic protein from the WWC family of proteins– was identified in 2003 in the human brain and kidney and has recently been associated with memory performance and synaptic plasticity. Through genome-wide screening, a single nucleotide polymorphism (SNP) was detected in the ninth intron of KIBRA gene (T→C substitution) and was implicated in human memory and the underlying neuronal circuitry. This review presents a synopsis of the current findings on the effects of the KIBRA SNP on human memory and synaptic plasticity. Overall the findings suggest impaired memory performance and less efficient or impaired hippocampal/medial temporal lobe (MTL) activation in CC homozygotes (in comparison to T carriers) with some differences between young and older subjects. This review also highlights limitations and potential sources for variability of studies' imaging findings along with future perspectives and implications for the role of KIBRA in memory-related brain systems.

Keywords: Cognition, episodic memory, fMRI, genetic polymorphism, KIBRA, synaptic plasticity.

INTRODUCTION

Memory is an indispensable cognitive trait allowing humans to encode, store and retrieve information. One form of long-term memory, declarative memory, is defined by the ability to consciously learn and recall facts and events and is further subdivided into semantic memory (the recollection of words, meanings and general concept-based knowledge involving factual information) and episodic memory (the recollection of personal/biographical events at a particular time and location in which the participant is at the centre of the episode) [1, 2]. This latter subtype was described as a major component of neurocognitive memory and the most vulnerable to neuropsychiatric disorders. In fact, impairments in episodic memory are a core deficit in a range of neurodegenerative diseases such as Alzheimer's disease, Huntington's disease and Schizophrenia [3, 4].

Historically, the study of Henry Gustav Molaison (patient HM) revolutionised the understanding of human memory and allowed the clear confirmation of the central role of the medial temporal lobe (MTL) and the hippocampus in declarative memory [5] with these two areas since being linked as part of a larger MTL declarative memory system [6]. The use of neuroimaging techniques has more recently helped to further investigate brain structures involved in memory processing, with fMRI studies providing evidence for the involvement of several brain regions in episodic

memory including the MTL, parietal lobe and frontal regions [7-9]. Thus, thanks to convergent lesion and neuroimaging studies, it is now well established that the MTL plays an essential role in the formation and the retrieval of new declarative memories and more specifically episodic memories [3, 10-13].

As a major prerequisite for genetic studies, episodic memory has to be heritable. Indeed, heritability of cognitive abilities, including memory performance, has been demonstrated in several studies on identical twins such as a study by McClearn *et al.* who detected a significant genetic influence on cognitive performance. In line with other twin studies, heritability was found to have a major influence on episodic memory performance [14, 15]. Therefore, as certain genes seem to account for specific phenotypic variance, these results could provide a first step towards understanding the pathways between genotypes and cognitive performance [14].

The combination of human genetic studies, brain imaging, and cognitive testing in previous studies has enabled the identification of several genes involved in synaptic plasticity and memory. For example, studies looking into the role of the human brain derived neurotrophic factor (*BDNF*) gene have found a strong association between this gene and the regulation of memory-related synaptic plasticity in the hippocampus [16-21] as well as apoE4 amongst others [22]. Although the association of genetics and cognitive performance helps us to understand how memories are formed, a further aim of these studies is to identify genetic risk factors for various neurodegenerative diseases such as Schizophrenia [17] and Alzheimer's disease

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[18, 19]. Thereby, human genetics studies on memory impaired patients might help to suggest that proteins such as BDNF and the protein of interest: Kidney and brain expressed protein (KIBRA) (and their related interactive proteins) might present potential pharmacological targets for neurodegenerative diseases such as Alzheimer's disease [23-26].

This review will highlight the current findings on the KIBRA single nucleotide polymorphism (SNP), memory and synaptic plasticity and aims to further provide possible explanations for the variability found within the results in current literature.

IDENTIFICATION OF THE KIBRA PROTEIN

First characterised by Kremerskothen *et al.* in 2003, KIBRA is a postsynaptic scaffolding protein located in the perinuclear region of the cytoplasm – more specifically in the hippocampal formation. In this initial study, KIBRA was first shown to interact *via* its protein domain-WW- with Dendrin (a postsynaptic protein involved in synaptic plasticity by modulating the structure of the synaptic cytoskeleton) [27]. In humans, a SNP in the *KIBRA* gene (rs17070145) leading to a T→C substitution in the ninth intron of the *KIBRA* gene has been linked to memory performance [25]. Several studies have suggested that the rs17070145 SNP, is associated with altered cellular and cognitive function with additional evidence for a higher risk of late-onset Alzheimer's disease – see below and earlier review by Schneider *et al.* [23].

KIBRA, encoded by the *WWCI* gene, was characterized as part of the WW domain-containing proteins family as it possessed known protein domains important for interactions with various other proteins. In a yeast two hybrid screen using a human isoform of Dendrin/KIAA0749, Kremerskothen *et al.* (2003) isolated a cDNA coding for the 'K7' protein [27]. Biochemical research using Northern Blot analysis was used to determine the expression of 'K7' mRNA in various adult tissues with results revealing predominant expression in the kidney and brain – hence the transcript 'KIBRA'. This mRNA codes for a cytoplasmic 125kDa protein that contains two amino-terminal WW domains at its N-terminus – responsible for binding to proline-rich sequences (PPxY), an internal motif similar to the C2 domain of synaptotagmin and a carboxy-terminal glutamic acid-rich stretch. Moreover, in order to examine the cellular function of KIBRA, yeast hybrid screens have been performed to detect other KIBRA-interacting proteins. In addition to Dendrin, protein kinase C (PKC) has been found to interact with KIBRA by phosphorylation of the protein at its C terminus as well as Synaptopodin [28, 29] and protein interaction with C-kinase 1 (PICK1) [30]. Further research on rat and human brains has demonstrated the expression of KIBRA in neurons of the hippocampal formation supporting a potential function of KIBRA in learning and memory. However, KIBRA does not seem limited to memory function as expression has also been observed in other brain regions unrelated to memory including the hypothalamus [29]. Furthermore, KIBRA has been found to be an upstream regulator of the Salvador/Hippo/Warts network, which controls tissue growth, in *Drosophila* [31, 32] and in humans [33, 34].

KIBRA AND ITS CONTRIBUTION TO SYNAPTIC PLASTICITY

Molecular studies have provided evidence for a possible role of PKC, PICK1, and Dendrin in synaptic plasticity, memory formation and long-term potentiation (LTP), explaining the increasing focus on the role of KIBRA in memory [27, 35]. Two of the strongest pieces of evidence connecting KIBRA to plasticity are its interaction with PKC ζ /PKM ζ [28, 36] and PICK1 [30]. Indeed, PKC is crucial in neuronal plasticity [37] and was found to co-localize and interact with KIBRA in the hippocampus and dentate gyrus [28]. Localisation of KIBRA within neurons was examined using co-immunostaining with antibodies and revealed high expression at the postsynaptic region of hippocampal neurons similar to Dendrin and Synaptopodin. PKM ζ is the active form of PKC ζ and Sacktor *et al.* first suggested their role in the maintenance of LTP [38]. Later, animal studies showed that PKM ζ activity in the hippocampus was both essential and sufficient to enhance synaptic transmission during LTP maintenance [39, 40]. This process is thought to occur through the modulation and trafficking of AMPA receptors [41, 42]. However, it should be noted that Volk *et al.* (2013) recently found that PKM ζ is not necessary for synaptic plasticity, learning, and memory [43]. Despite this one finding, multiple pieces of evidence lead to the hypothesis that KIBRA may recruit postsynaptic proteins such as Dendrin to modulate postsynaptic cytoskeleton structure, important for synaptic plasticity [44]. These interactions and localisation findings provide a strong indication of KIBRA's potential critical role in postsynaptic processes that regulate learning and memory formation.

A study done by Makuch *et al.* (2011) demonstrated that KIBRA has a role in the modulation of AMPA receptor membrane trafficking and recycling which is critical for synaptic plasticity [30]. The authors found that KIBRA is a direct binding partner of PICK1, which is an important factor in regulating AMPA receptor trafficking [45-47]. Moreover, knock-out of *KIBRA* in adult mice resulted in both a reduction of long-term potentiation and long-term depression in hippocampal synapses, as well as learning and memory defects in the form of a significant reduction in the retention of fear memory following fear conditioning compared to wild-type [30]. Thus, although the molecular function of KIBRA is not completely understood, several studies have demonstrated that KIBRA not only modulates membrane trafficking and perhaps cytoskeleton structure of hippocampal neurons through its interaction with various proteins involved in synaptic plasticity but also is critical in behavioural learning and memory. These interactions in combination with animal studies support the hypothesis that KIBRA plays an important role in the regulation of synaptic plasticity and memory.

However, to our knowledge, no clinical studies have been performed to directly assess the impact of *KIBRA* rs17070145 using physiological measurements of synaptic plasticity. The closest instance was a study done by Witte *et al.* that utilized a neurostimulation technique to induce motor cortex plasticity in a cohort of 2 X 16 healthy young females (age: $M = 27$, $SD = 8$) [48]. Through the use of a

transcranial magnetic stimulation (TMS) and paired associative stimulation (PAS) protocol, the authors evaluated the effects of the *BDNF* Val66Met genotype on motor cortex plasticity while also taking KIBRA rs17070145 carrier status into account. The *BDNF* and *KIBRA* genotypes were found to have no significant impact on TMS and PAS-induced plasticity. Therefore, despite the *in vitro* and *in vivo* research that suggests *KIBRA*'s involvement in synaptic plasticity, they found no evidence to support this theory. Nevertheless, a degree of caution should be applied to this finding, as the primary gene polymorphism studied was *BDNF* and not *KIBRA*. While additional neurostimulation studies that directly measure the effect of *KIBRA* rs17070145 on synaptic plasticity are needed, another method to evaluate *KIBRA*'s role in regulating synaptic plasticity is to look at its effect on memory performance.

THE ROLE OF KIBRA IN EPISODIC MEMORY IN HEALTHY SUBJECTS

A) Behavioural Studies

The link between KIBRA and cognition was made following a genome-wide association study by Papassotiropoulos *et al.* [25] reporting an association between the KIBRA SNP and memory performance in two Swiss cohorts and one American cohort. The main SNP reported (rs17070145) was a T→C substitution in the ninth intron of KIBRA, which was found to show differences in memory performance depending on the genotype of participants. To assess behavioural differences, a memory test was used involving the learning and immediate and delayed (5 min and 24 hours) recall of unrelated nouns. They found that CC homozygotes carriers had significantly worse episodic memory performance (less words recalled after both delays) compared to T carriers ($P < 0.001$). As TT had a lower frequency and showed similar performance in several memory tests to CT genotypes, both genotypes were combined for the analysis as "T carriers" for statistical analysis. Control tasks were performed to check for confounding factors such as concentration and attention and no allele-dependent associations were found for those tasks. These behavioural results were also confirmed in the second Swiss and American cohorts [25].

Following the initial publication, several studies have examined the effect of the KIBRA polymorphism on episodic memory performance of healthy participants and memory-impaired patients in an attempt to replicate the findings of Papassotiropoulos *et al.* [25]. Overall, studies recruiting healthy participants have consistently found worse episodic memory performance in CC homozygotes [24, 26, 49-52] except one study, which did not find any association [53] (see Table 1).

In fact, the first replication study tested a small cohort (N=64) of healthy elderly subjects (mean age: 67) on the Verbal Learning and Memory Test (VLMT) consisting of a free recall task of a list of nouns immediately and after a delay of 30 minutes as well as a recognition task. CC homozygotes showed poorer performance in recall and recognition compared to T carriers [26]. Larger studies yield similar results in 312 healthy elderly participants (mean

age=71) using an immediate and delayed recall and recognition episodic memory test [24] and in 383 healthy young adults (mean age=26) using an item-pair memory task (learning of single words and pair of words) with item recognition and associative recognition phases [50]. Similarly, in the largest study performed to date in two Scottish cohorts of healthy elderly participants (N=2091 and N=542), with recall and recognition of list of unrelated words (Rey Auditory Verbal Learning Test) and the immediate and delayed recall of stories (Wechsler Logical Memory Test), CC homozygotes were found to have worse performance (compared to T carriers) in delayed recall task of unrelated words but not for the recall of prose [51]. Finally, Need *et al.* [53] failed to find any effect of the polymorphism on verbal recall and story immediate and delayed recall which partially replicate the findings of the study by Bates *et al.* (2009) [51]. Although the sample size was similar to previous studies, Need *et al.* [53] study combined differently their genotype groups-CC and CT genotypes together- which could account for the null results.

Therefore, although these studies have used different age and ethnicity cohorts as well as variable episodic memory tests, most studies have shown a consistent effect of the KIBRA SNP on memory performance with CC homozygotes showing impaired performance on declarative memory with findings generalizable across populations and cognitive tasks. These overall results have been confirmed in two meta-analyses which assess the behavioural effect of the KIBRA SNP. The first of these meta-analyses incorporated 8000 subjects from five studies examined the association of KIBRA T allele with Alzheimer's disease risk by calculating an overall odds ratio estimate. A marginal protective risk estimate for the T allele was concluded (OR=0.94; $p=0.07$) [54]. A second meta-analysis included 12 studies with a sample size of N=8909 and investigated the correlation between behavioural variance and genotypic variation between subjects. The study observed an overall effect size of 0.5% explained variance (correlation 42 coefficient $r=0.068$, $p=0.001$) but did not investigate the direction of the effect [55].

B) Imaging Studies

In the initial report by Papassotiropoulos (2006), functional magnetic resonance imaging (fMRI) was also used to measure brain activation using blood-oxygen-level dependent contrast (BOLD) signal (hereafter referred to as 'activation') in the whole brain and regions involved in memory formation including the hippocampus. Brain activation was measured in a subgroup of healthy young subjects (30 subjects, age: 19-25 years) from the first Swiss cohort [25]. A face-profession paired associative learning task -measuring episodic memory- was given to participants while recording BOLD signal with fMRI. During the learning phase, each pair consisted of a face associated with a profession and subjects were asked to imagine a scene in which the person practices his attributed profession and to report the difficulty of imagining the scene (easy or hard). For the retrieval phase, the same faces were presented again, without the professions, and subjects were asked to recall the

Table 1. Summary of studies associating KIBRA and episodic memory in healthy subjects.

Study (Author/Year)	Sample Information	Cognitive / Memory Testing	Imaging	Results	Conclusions	
HEALTHY PARTICIPANTS						
Papassotiropoulos <i>et al.</i> 2006	Healthy subjects Swiss: N=351; 18-48 years old Swiss: N=428; 18-28 years old USA: N=256; 20-81 years old	Unexpected delayed free-recall: test of learned words.	Yes (10 males, 20 females, median age 22); face-profession paired for associative learning; tested hippocampal activation.	TT/CT -Behavioural: better free recall and memory performance in various episodic memory test in 3 independent populations.	CC -fMRI: increased hippo, MTL, frontal, parietal cortex activation	Non-carriers of T allele need more activation for the same level of retrieval performance as T carriers. Potential role for KIBRA in human memory, specifically episodic memory.
Almeida <i>et al.</i> 2008	Healthy and partial Mild cognitive impairment (MCI) Caucasian: N=312; over 50 years old	IQ reading test; CERAD (mixture of test, include episodic memory)	No	TT/CT	CC	Older healthy adults with CC genotype have worse episodic memory than carries of the T allele. KIBRA gene does not play a significant role in the phenotypic expression of amnesicMCI.
					<u>Healthy group</u> : Lower scores in episodic memory tasks.	
				<u>MCI group</u> : No obvious associations between KIBRA genotype and memory scores		
Need <i>et al.</i> 2008	2 cohorts: Duke genetics of memory, N=319 German cohorts, N=365	Verbal recognition memory, AVLT, immediate an delayed recall of stories.	No	No association between T allele and immediate/delayed recall.	Failed to show association between KIBRA allele and memory performance. Failed to replicate the results found in Papassotiropoulos	
Bates <i>et al.</i> 2009	Scottish (55-82 years old); from a trial for aspirin for asymptomatic atherosclerosis, (represents general population). Second sample: members from Lothian birth cohort 1921, normal ageing population.	Immediate & delayed memory with 5 learning trials, for the second sample: Wechsler logical memory test.	No	KIBRA T carrier status significantly affected delayed recall	Suggests an effect of KIBRA of modest size and specific to forgetting over the delay interval. The effects of KIBRA on episodic memory appear in this sample to be modest and specific for a particular cognitive component of memory, related to item-based recall rather than affecting a broad range of memory phenotypes.	
Preuschhof <i>et al.</i> 2010	Healthy volunteers Caucasian origin N=383 (20-31 years old)	Tests: executive functioning), spatial working memory, fluid and digital intelligence, cognitive abilities Episodic memory tasks: item-pair memory task.	No	TT/CT	CC	Confirmed findings of Papassotiropoulos. KIBRA and CLSTN2 have an interactive positive effect on memory performance. Results also showed an increased positive effect of KIBRA T allele in more demanding tasks.
				Higher episodic memory performance Enhanced effect if subject is CLSTN2 C carrier		

Table 1. contd....

Study (Author/Year)	Sample Information	Cognitive / Memory Testing	Imaging	Results	Conclusions	
HEALTHY PARTICIPANTS						
Kauppi <i>et al.</i> 2011	Non-demented patients Swedish: N=2230; 35-85 years old from Betula project second wave (for cognitive data) N=113; 55-80 years old (for fMRI)	4 tests of immediate free recall of words: 1) encoding; 2) retrieval; 3) encoding and retrieval; 4) no distraction; delayed cues recall; block design score. Scanner task: face-name paired-associate task	Yes, 3 samples: N=83; 55-60 y old; N=64; 55-60 years old; N=113; 65-75 years old Tasks: face-name paired-associate task; 3 conditions tested: encoding, retrieval, control. fMRI examined hippo/MTL activation.	TT/CT	CC	Increased episodic memory performance in T carriers. Increased hippocampal activation relate to faster response times during retrieval. Conclusion: superiority of T carrier in episodic memory is mediated through improved hippocampal functioning (contrary to the conclusion of Papassotiropoulos study).
				-Behavioural: Better episodic memory performance in immediate free recall and scanner task (episodic memory). -fMRI: higher hippo/MTL activity associated with faster response time but not recall accuracy.		

profession associated with the face and to determine the category: academic or workman. The same baseline was used for both phases of the task (encoding and retrieval), which required the subject to determine the biggest ear of a head contour [25]. In the absence of a retrieval performance difference between genotype groups, CC homozygotes were found to have significantly higher brain activation in the MTL, specifically the hippocampus and parahippocampal gyrus, compared to T carriers during memory retrieval (but no differences during encoding) [25]. These findings were interpreted as CC homozygotes needing higher hippocampal activation to attain similar levels of retrieval performance as T carriers (i.e. CC homozygotes having a less efficient brain network to perform the task). Such an increase in activation has been suggested to reflect compensatory process [56]. The less efficient processing (i.e. compensation) observed when behavioural performance is matched supports behavioural studies which overall showed impairment in declarative memory in CC homozygotes.

A more recent study in elderly non-demented subjects (N=113 for fMRI study) used fMRI association with a face-name paired associate task [49]. Similarly to other behavioural studies, TT and CT subjects showed better performance in episodic memory tasks outside the scanner than CC homozygotes, although the behavioural difference was only seen for immediate free recall of words. Performance for the scanner task differed between both groups with T carriers performing better than CC homozygotes. Regarding the imaging results, the study showed differences in hippocampal activation between the groups with CC homozygotes showing lower MTL (activation associated with slower response times) solely during retrieval. This activation difference remained following *post hoc* group matching for task performance. These findings in elderly subjects are in contrast to that reported in young subjects where the opposite pattern of brain activity changes were reported [25]. In

contrast to the original study where an increase in hippocampal activity in CC homozygotes was interpreted as “less efficient” brain activity during cognitive performance, Kauppi *et al.* [49] interpreted this decreased activation in CC homozygotes as a reflection of impaired hippocampal functioning. These studies highlight the issues associated with functional imaging studies, especially inconsistencies in the interpretation of BOLD activations. It is possible that in young people (as in the Papassotiropoulos *et al.* study), the increased activity is a compensatory response and that in elderly subjects (as in the Kauppi *et al.* study) this compensatory response is impaired (i.e. possibly related to deficient cognitive reserve), leading to a decrease in activity. However, in both cases, the changes potentially reflect an inefficient brain response during cognitive processing in the CC homozygotes.

These studies suggest consistent pattern of findings across behavioural and imaging studies with CC homozygotes showing less efficient MTL/hippocampal activation and memory impairments. However, further work is needed to clarify the exact relationship between changes in brain activation and memory performance given the opposite pattern of activation observed in young and elderly subjects. Furthermore, there are a number of methodological factors that need to be taken into consideration.

A common methodological flaw in both previous fMRI studies looking at the effect of the KIBRA SNP on memory-related neural activation is the lack of correction for multiple comparisons and the arbitrary p value chosen as the threshold for activation, which could account for the conflicting results. In particular, inconsistent results may be due to variable approaches to controlling for Type 1 error (voxels activated above threshold by chance) across studies. Kauppi *et al.* and Papassotiropoulos *et al.* performed uncorrected statistics with different minimum cluster sizes of

significance and statistical threshold, (critical p values of 0.01 and 0.001, respectively). Although both studies focused on MTL activation, this area of the brain comprises thousands of voxels, which are compared simultaneously. Therefore, even with a relatively conservative threshold, this large amount of uncorrected statistical tests may lead to some voxels activated by chance.

As fMRI analysis involves multiple comparisons across thousands of voxels, lack of correction will lead to an inflated type I error. The best practice to correct for these numerous statistical comparisons is by modifying the significance level (p-value) to account for the number of comparison made in the study [57]. When restricting the fMRI analysis to a brain region of interest, one accepted way to do this is using small volume correction (corresponding to the number of voxels compared) with the family wise error (FWE) rate (type I error rate) [58]. However, it is also important to consider that adopting the most conservative approach (FWE rate) may lead to increasing the rate of type II errors (false negatives) [59].

Variability may also be due to the age difference in the cohorts recruited for both studies. In fact, Kauppi *et al.* [41] focused on an elderly cohort (55-75 years old) whereas Papassotiropoulos *et al.* [21] looked at a young healthy cohort (19-25 years old). Thus, the opposite fMRI results may be accounted for a potential age-dependent effect of the KIBRA polymorphism on hippocampal activation.

Finally, a recent imaging study has focused on the hippocampal volume differences in female T carriers and CC homozygotes and have detected volume differences between the two groups in the *Cornu Ammonis* areas and dentate gyrus which may be linked to a neural correlate of the effects of KIBRA SNP on cognition. However, this study needs to be replicated in a larger and more diversified cohort (including more males) using various brain segmentation approaches as the method used did not differentiate well hippocampal subregions.

CONCLUSION

KIBRA, first related to memory in 2003 through human genome-wide screening, has become an attractive target for research related to learning, memory, and synaptic plasticity. Molecular investigations have established multiple KIBRA interaction partners, many of which play key roles in the trafficking of molecules important to synaptic plasticity. Moreover, behavioural and imaging studies such as the seminal paper by Papassotiropoulos *et al.* found significantly worse declarative memory performance in KIBRA CC homozygotes as compared to T carriers. These findings have largely been replicated in behavioural independent studies. Furthermore, functional imaging findings support these behavioural observations by showing inefficient or impaired brain activation in CC homozygotes. Given the paucity of studies to directly measure and test KIBRA's impact on synaptic plasticity, additional research is needed. In particular, areas to focus on include studies in humans using markers of synaptic plasticity and studies (both animal and human) examining the relationship between changes in synaptic plasticity and memory. Nevertheless, the molecular basis between KIBRA and synaptic plasticity certainly

provides credibility to KIBRA's influence on human memory performance. The findings, to date, suggest that the KIBRA polymorphism might have an impact on plasticity and memory. However further exploration in both animals and humans are needed to gauge the true impact of KIBRA on learning, memory, and synaptic plasticity.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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