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The KIBRA Gene: Learning About Memory

Danielle Clark
Lake Forest College

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Danielle Clark*
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Summary

Memory is a polygenic trait that enables humans to learn and remember information. A novel genome-wide association study performed in 2006 implicated that a polymorphism in the KIBRA gene was associated with enhanced episodic memory. Very little detail is known about the pathways to which this gene contributes; however, research implicates that the cytoplasmic protein plays a structural role in neurons. Recent studies examining the gene's association with memory performance are consistent and age-independent in healthy individuals; however, conflicting results when examining subjects with impaired memory suggest KIBRA's effects are complex. Although one memory-enhancing therapy has suggested based upon these findings, future research must focus on the genetic and molecular mechanisms through which KIBRA acts. Greater understanding of these pathways will allow for the discovery and understanding of therapies that can potentially treat non-disabling memory declines associated with healthy aging, as well as impaired memory associated with diseases such as Alzheimer's.

Introduction

Memory is a fundamental cognitive ability that enables humans to learn and remember information. This polygenic trait gives meaning to people's lives by providing context and understanding to daily events. Episodic memory, which refers to the encoding and retrieval of facts that occur at a specific time, is thought to have a heritability of approximately 50 percent (McClearn et al., 1997). Ascertaining the genetic aspects of this ability could provide promising targets for memory-enhancing pharmaceuticals. These drugs could play a role in the treatment of non-disabling memory declines associated with healthy aging. Furthermore, these medications could act as potential treatments for patients with impaired memory, such as those with Alzheimer's disease, other dementias, or mild cognitive impairment. The genetic information behind memory could theoretically be used to improve the lives of approximately 5.2 million people in the United States living with Alzheimer's disease (www.alz.org).

Recent technological advances have altered geneticists' inquiry of memory, for whole-genome association studies allow for the identification of specific points of variation in human DNA that underlie particular diseases or traits (www.genome.gov). Identifying the genetic factors that influence memory is central to discovering and developing medicines that target diseases with increased precision and reduced risks. One such novel study carried out in 2006 reported an association between a polymorphism in the KIBRA gene and episodic memory. Before this discovery is illuminated, however, one must first examine the scientific history of the gene itself.

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Discovering and Characterizing KIBRA

In 1998, Nagase *et al.* reported the sequences of 100 cDNA clones from a set of size-fractionated human brain cDNA libraries. The scientists of the Kazusa DNA Research Institute focused their sequencing efforts on the analysis of large cDNAs, which they defined as over 4 kb. Clones were then selected for sequencing if the protein product was also large (over 50 kDa). One of the 100 sequences described was KIAA0869, which was further classified as 3,408bp long with an open reading frame (ORF) length of 888 amino acid residues. The gene was found to be located on chromosome 5 using the UniGene database. Furthermore, using RT-PCR ELISA, the scientists found KIAA0869 was highly expressed in the liver and kidney, while intermediate expression was detected in the brain, lung, pancreas, and ovary. Weak expression was observed in the heart and testis, and no expression was found in skeletal muscle and spleen (Nagase et al, 1998).

Almost five years later, the actual structure of KIAA0869 was elucidated. Kremerskothen *et al.* further characterized the gene in 2003, providing its current names based upon their findings: WW, C2, and Coiled-Coil Containing 1 (WWC1), also known as Kidney and Brain Expressed Protein (KIBRA). The gene is more commonly referred to by the latter term KIBRA. Using a yeast two hybrid screen with the human Dendrin protein as bait, Kremerskothen *et al.* (2003) isolated what they at first entitled K7 due to its strong activation of reporter genes. They found that K7 contained a cDNA of approximately 4.1kB with an ORF encoding a 1113 amino acid protein. Furthermore, Kremerskothen *et al.* (2003) identified that the sequence of K7 starting at amino acid 224 was identical to the KIAA0869 sequence described by Nagase *et al.* (1998). Through hybridization of cDNA to genomic fragment, the gene was mapped to chromosome 5q34-5q35.2 (OMIM, Kremerskothen *et al.* 2003).

Once Kremerskothen *et al.* (2003) identified the protein, they set forward to further characterize the structure; a representation is shown below in Figure 1. These researchers correctly hypothesized that the hydrophilic protein was cytoplasmic, for it contained neither signal sequences nor hydrophobic stretches to implicate a role in membrane signaling. Two amino-terminal WW domains, the smallest globular domain known to mediate protein-protein interactions by binding to proline-rich sequences, were identified; by using mutated fusion proteins in a phage ELISA, the interaction between the WW domains and target proteins was found to be predominantly mediated by the first WW domain. The binding specificity of the second WW domain remains unknown (Kremerskothen *et al.*, 2003). The researchers also identified a C-terminal glutamic acid-rich stretch as well as a C2-like domain, implying that K7 is a Ca^{2+}-binding protein (Kremerskothen *et al.*, 2003). A Northern blot was performed to examine the expression of K7. Because the 4.3kB transcript was highest in kidney and brain, Kremerskothen *et al.* (2003) called the transcript KIBRA. The cellular distribution of the transcript within the cytoplasm was later confirmed by analyzing green monkey kidney cells transfected with plasmids that encoded Myc-tagged KIBRA. Kremerskothen *et al.* (2003) also determined that mutants lacking the WW domains were still expressed only in the cytoplasm. Because there was no difference in the distribution of full-length and truncated KIBRA, the WW domains (or the interactions they direct) are not necessary for correct localization.

Figure 1. A schematic representation of the KIBRA protein adapted from Traer *et al.* (2007).

The Function of KIBRA

Little is known about the specific function of KIBRA aside from which proteins it interacts with. Kremerskothen *et al.* (2003) postulated that the protein plays a structural role in the cell. This hypothesis is supported by KIBRA's interaction with Dendrin, a protein associated with the cytoskeleton. Similar to this study, much of the other known information about the functional properties of KIBRA has been uncovered while studying other proteins. For example, one research group set out to determine the function of KIAA0513 and the gene's relationship to schizophrenia (Lauriat *et al.*, 2006). Using a two-hybrid screen, KIBRA was found to interact with the protein product of this gene, which is suspected to be involved in signaling pathways related to neuroplasticity. Similarly, KIBRA was found to complex with dynein light chain 1 (DLC1) when researchers set out to identify DLC1's pathway. This association seems essential for estrogen receptor-α transactivation in breast cancer cells and is thought to play a mechanistic role in conferring an optimal ER transactivation function as well as the proliferation of these cells (Rayala *et al.*, 2006). This hypothesis, however, has not yet been confirmed.

Another binding partner of KIBRA, protein kinase C ζ (PKC-ζ), was identified by Buther, Plaas, Barnekow, and Kremerskothen in 2004. This protein plays a significant role in signal transduction processes, including the formation of memory. The study suggests that PKCζ phosphorylation may regulate the cellular function of KIBRA by phosphorylating the WW domain at the C-terminus (Buther, Plaas, Barnekow, & Kremerskothen, 2004). Although these binding partners of KIBRA have been identified, the specific functions of the gene in the pathways are not yet understood.

KIBRA and Memory

Aside from the basic structure and associated proteins, however, KIBRA was not well studied until a press release in 2006; the Translational Genomics Research Institute (TGen) announced the discovery of a gene that "plays a significant role in memory performance in humans" (TGen, 2006). The study was the first to describe scanning the human genetic blueprint to identify cognitive differences between humans; Passotiropoulos *et al.* (2006) stated that a genome-wide screen with over 500,000 SNPs using microarrays showed that a single nucleotide polymorphism (SNP) within the ninth intron of the KIBRA gene was significantly associated with memory performance in three independent subject groups. An initial screen of 341 young adults in Switzerland were separated into four groups based upon their verbal memory performance and were consequently genotyped for 502,627 SNPs. Subjects with the SNP rs1707145 T allele of KIBRA had 24% better recall five minutes after word presentation and 19% better recall 24 hours later than non-carriers. To verify these results, similar tests were performed on a second, median-aged cohort in the United States, as well as a third population of young adults from Switzerland (Passotiropoulos *et al.*, 2006). Subjects heterozygous or homozygous for the T allele had better memory performance than non-carriers (CC allele).

While Passotiropoulos *et al.* (2006) demonstrated the association of the rs1707145 T allele with better episodic memory in young and middle aged adults, a study performed in 2008 used healthy elderly subjects to replicate the findings (Schaper, Kolsch, Popp, Wagner, and Jesseen, 2008). This study ensured that age did not affect the role the SNP allele had on recall performance.

Furthermore, Passotiropoulos *et al.* (2006) showed that KIBRA was expressed in memory-related brain structures (such as the hippocampus), and fMRI studies of the three cohorts detected allele-dependent differences in hippocampal activations during memory retrieval, supporting KIBRA's connection to memory. Johannsen, Duning, Pavenstadt, Kremerskothen, and Boeckers (2008) similarly found that KIBRA was expressed in the hippocampus, cortex, hypothalamus, and cerebellum. They propose that KIBRA has a diverse role in memory-related and neuroendocrine mechanisms, including brain development and memory formation by acting as a postsynaptic scaffold protein connecting cytoskeletal and signaling proteins.

Almeida *et al.* (2008) also ensured that the polymorphism's association with memory performance, especially in the elderly, was related to episodic memory and not the decreased expression of the mild cognitive impairment (MCI) phenotype. Those with MCI, a trait defined as an increased risk of developing dementia as one ages, often show normal age-adjusted cognitive abilities but display significant deficits in tests of episodic memory. Because the genetic determinants of MCI and dementia may not be identical, the authors suspected that the KIBRA polymorphism could play a role in the development of MCI later in life. This hypothesis, however, was unsupported; although the results replicated those of Passotiropoulos *et al.* (2006) and Almeida *et al.* (2008) regarding episodic memory enhancement, no statistically significant evidence was found suggesting CC carriers were at higher risk for MCI (although a slight trend in this direction was observed).

KIRBA and Memory Disorders

Since the T allele of the KIBRA gene is associated with improved episodic memory in all ages, Rodriguez-Rodriguez *et al.* (2009) set out to determine if the polymorphism conferred protection to Alzheimer's disease (AD). AD is known to not only impair episodic memory but adversely affects hippocampal function, a structure that shows high expression of KIBRA. The study examined 391 subjects who met criteria for probable sporadic AD, but the results were inconsistent with an association of allelic differences between the AD subjects and controls. However, when the

AD subjects were stratified by age of onset, a significant correlation was found for the T allele of rs17070145 and increased susceptibility of AD in the very elderly (classified as over 86 years of age). This result conflicts with previous memory studies in healthy individuals that showed age-independent effects of KIBRA alleles (Passotiropoulos *et al.*, 2006; Schaper, Kolsch, Popp, Wagner, and Jesseen, 2008). Rodriguez-Rodriguez *et al.* (2009) propose that different genetic backgrounds of late- and early-onset patients may enhance the effect of the KIBRA gene, leading to a greater susceptibility for late expression of the disease.

These findings also contradict a study in which non-carriers of the T allele have a moderate but significantly increased risk of AD; Corneveaux *et al.* (2008) suggest that the T allele may have a protective effect with progression increasing later age-of-onset. These opposing results may be due to differences in power, for the study performed by Corneveaux *et al.* (2008) used 2.5 times the number of subjects, including relatively error-free postmortem tissue. Unlike this sample, Rodriguez-Rodriguez *et al.* (2009) used subjects from an isolated region in Spain.

Corneveaux *et al.* (2008) also utilized microarray to examine differences in expression levels of KIBRA and four known binding partners, hypothesizing that the KIBRA pathway would exhibit deregulation in the brains of AD patients. Compared to controls, KIBRA was over-expressed while three of the four binding partners were under-expressed in three areas of the brain known to be affected by AD. Furthermore, PET scans suggested an association between noncarriers of the T allele and glucose metabolism in certain brain regions known to be metabolically affected by AD (Corneveaux *et al.*, 2008).

Due to the opposing effects of rs17070145 alleles on episodic memory and AD, Nacmias *et al.* (2008) chose to examine the relationship between the KIBRA SNP and subject memory complaints (SMC), or problems with everyday memory. Their study aimed to identify an association between SMC, which are common in older adults, while taking into account the ApoE genotype as it has a selective effect on episodic memory, attentional functions, and hippocampal volumes in both healthy and AD subjects (Hashimoto, 2001). SMC subjects with the CT/TT genotype did not perform as well as those with the CC genotype on long-term memory tests. Although a small sample size may have contributed to differences in results from this study and its predecessors, Nacmias *et al.* (2008) advocate that this contradicting data suggests KIBRA's role is quite complex; the KIBRA genotype may affect memory performance differently in healthy subjects compared to those with memory deficits (AD or SMC). This hypothesis supports the unexpected results of Rodriguez-Rodriguez *et al.* (2009), for the SNP was associated only in very elderly AD patients; however, studies of healthy subjects found that the association was age-independent (Passotiropoulos *et al.*, 2006; Almeida *et al.*, 2008).

From Polymorhpism to Pharmaceuticals

The rs1707145 SNP in KIBRA has shown conflicting effects on memory. Although Nacmias *et al.* (2008) believes the polymorphism has varying effects depending upon the health of the individual, Huentelman *et al.* (2009) imply that prior results raise the possibility that a common molecular mechanism may contribute to both episodic memory and the pathological and clinical features of AD. The hypothesis of overlapping mechanisms drove these scientists to attempt to target specific processes for memory-enhancing and AD risk-reducing therapies. Based upon the previously mentioned findings and a pathway analysis approach, Huentelman *et al.* (2009) hypothesized a pathway that could be used to alter KIBRA activity. The RhoA/ROCK/Rac pathway was demonstrated to be upstream of PKC-ζ, one of KIBRA's binding partners. Furthermore, this pathway has been implicated in key neurobiological functions such as neurite outgrowth and other processes that underlie cognitive function. Based upon this knowledge, Huentelman *et al.* (2009) inhibited the RhoA/ROCK/Rac pathway in a rodent model using hydroxyfasudil to determine if enhanced memory and learning would ensue. Both spatial learning and working memory were improved in aged rats, implicating ROCK activity in these processes. Huentelman *et al.* (2009) state that one plausible mechanism may be phosphorylation of KIBRA by PKC-ζ after activation of Rac-1. However, this hypothesis must be confirmed by further experimentation. If substantiated, this drug may have clinical relevance as a cognitive enhancer, for the parent drug (Fasudil) is safe and well-tolerated by humans – it is currently used to treat stroke victims. More research must be done, however, in order to determine if only KIBRA is altered and not the molecules it interacts with.

Conclusion

The findings of Huentelman *et al.* (2009) support the quest for pharmaceuticals that could potentially treat cognitive impairment, cushion the impact of aging on memory, or even enhance learning and memory. KIBRA is associated with many other proteins in significant processes, such as episodic memory and late-onset Alzheimer's disease. However, the precise mechanisms through which KIBRA contributes to these pathways are not fully understood or confirmed; conflicting results must be resolved by further research. Overall, these results suggest that KIBRA's role in both episodic memory and AD is complex. Further examination of these mechanisms must occur, as this knowledge could allow for the discovery and understanding of therapies such as hydroxyfasudil. The findings of Huentelman *et al.* (2009) support the quest for pharmaceuticals that could potentially treat cognitive impairment, cushion the impact of aging on memory, or even enhance learning and memory. However, even this drug, although successful in improving memory in rats, is not yet understood.

References

Almeida, O.P., Schwab, S.G., Lautenschlager, N.T., Morar, B., Greenop, K.R., Flicker, L., et al. (2008). KIBRA genetic polymorphism influences episodic memory later in life, but does not increase the risk of mild cognitive impairment. Journal of Cellular Molecular Medicine, 12(5A), 1672-1676.

Alzheimer's Association. (2009). Retrieved March 14, 2009. http://www.alz.org/alzheimers_ disease_facts_ figures.asp

Buther, K., Plaas, C., Barnekow, A., & Kremerskothen, J. (2004). KIBRA is a novel substrate for protein kinase Cζ. Biochemical and Biophysical Research Communications, 317, 703-707.

Corneveaux, J.J., Liang, W. S., Reiman, E. M., Webster, J. A., Myers, A. J., Zismann, V. L. et al. (2008). Evidence for an association between KIBRA and late-onset Alzheimer's disease. Neurobiology of Aging, 10.1016/j.neurobiolaging.2008.07.014.

Hashimoto, M., Yasuda, M., Tanimukai, S., Matsui, M., Hirono, N., Kazui H., et al. (2001). Alipoprotein E epsilon 4 and the pattern of

regional brain atrophy in Alzheimer's disease. Neurology, 57(8), 1461-1466.
Huentelman, M., Stephan, D., Talboom, J., Corneveaux, J., Reiman, D., Gerber, J., et al. (2009). Peripheral delivery of a ROCK inhibitor improves learning and working memory. Behavioral Neuroscience, 123(1), 218-223.

Johannsen, S., Duning, K., Pavenstadt, H., Kremerskothen, J., & Boeckers, T.M. (2008). Temporal-spatial expression and novel biochemical properties of the memory-related protein KIBRA. Neuroscience, 155, 1165-1173.

Kremerskothen, J., Plaas, C., Buther, K., Finger, I., Veltel, S., Matanis, T. et al. (2003). Characterization of KIBRA: a novel WW domain-containing protein. Biochemical and Biophysical Research Communications, 300(4), 862–867.

McClearn, G., Johansson, B., Berg, S., Pedersen, N., Ahern, F., Petrill, A., et al. (1997). Substantial genetic influence on cognitive abilities in twins 80 or more years old. Science, 276(5318), 1560 – 1563.

Nacmias, B., Bessi, V., Bagnoli, S., Tedde, A., Cellini, E., Piccini, C., et al. (2008). KIBRA gene variants are associated with episodic memory performance in subjective memory complaints. Neuroscience Letters, 436, 145-147.

Nagase, T., Ishikawa, K., Suyama, M., Kikuno, R., Hirosawa, M., Miyajima, N., et al. (1998). Prediction of the coding sequences of unidentified human genes. XII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Research, 5, 355-364.

Nation Human Genome Research Institute. (2009). Whole Genome Association Studies. Retrieved March 16, 2009. http://www.genome.gov/17516714

Papassotiropoulos, A., Stephan, D.A., Huentelman, M.J., Hoerndli, F.J., Crai, W., Pearson, J.V., et al. (2006). Common Kibra alleles are associated with human memory performance. Science, 314(5798), 475–478.

Rayala, S., Hollander, P., Manahathi, B., Talukder, A., Song, C., Peng, S., et al. (2006). Essential role of KIBRA in co-activator function of dynein light chain 1 in mammalian cells. Journal of Biological Chemistry, 281(28), 19092-19099.

Rodriguez-Rodriguez, E. , Infante, J., Llorca, J., Mateo, I., Sanchez-Quintana, C. , Garcia-Gorostiaga, I., et al. (2009). Age-dependent association of KIBRA genetic variation and Alzheimer's disease risk. Neurobiology of Aging, 30, 322-324.

Schaper, K., Kolsch, J., Popp, M., Wagner, F., & Jessen, N. (2008). KIBRA gene variants are associated with episodic memory in healthy elderly. Neurobiology of Aging, 29, 1123-1125.

The Translational Genomics Research Institute Press Release. (October 19, 2006). Research team identifies human "memory gene."

Traer, C., Rutherford, A., Palmer, K., Wassmer, T., Oakley, J., Attar, N., et al. (2007). SNX4 coordinates endosomal sorting of TfnR with dynein-mediated transport into the endocytic recycling compartment. Nature Cell Biology, 9(12), 1370-1380.