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Introduction

Memory is a fundamental cognitive ability that enables humans to learn and remember information, providing meaning and context 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). A whole-genome association study in 2006 identified an association between a single nucleotide polymorphism (SNP) in the KIBRA gene and episodic memory (Passotiropoulos *et al.*, 2006). This one genetic aspect of memory provided a promising target for memory-enhancing pharmaceuticals. Huentelman *et al.* (2009) used hydroxyfasudil to inhibit the RhoA/ROCK/Rac pathway in a mouse model. This pathway had been previously reported to be upstream of PKC-ζ, one of KIBRA's binding partners. Using this pharmaceutical, Huentelman *et al.* (2009) observed spatial learning and working memory improvement in aged rats, suggesting a possible therapy to treat cognitive impairment such as that seen in Alzheimer's disease, cushion the impact of aging on memory, or simply enhance learning and memory. However, the associations between KIBRA, memory, and this drug have not yet been elucidated.

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 (Kremerskothen et al., 2003). Multiple studies examining the gene's association with memory performance found consistent and age-independent results in healthy individuals (Passotiropoulos *et al.*, 2006; Schaper, Kolsch, Popp, Wagner, and Jesseen, 2008); however, conflicting results when examining subjects with impaired memory suggest KIBRA's effects are complex and may even be involved in Alzheimer's disease (Rodriguez-Rodriguez *et al.*, 2009; Corneveaux *et al.*, 2008). The precise mechanisms through which KIBRA contributes to these pathways will only be understood through further research. Therefore, a conditional knockout of KIBRA in a mouse model should be created to better understand the role KIBRA plays in memory. This pathway, once elucidated, will confirm KIBRA's association to memory and the drug hydroxyfasudil, as well as allow for the discovery and understanding of therapies that can potentially treat either non-disabling memory declines associated with healthy aging and impaired memory associated with diseases such as Alzheimer's.

Specific Aims

1. The first goal is to produce a conditional knockout mouse of the KIBRA gene using the Cre/loxP system and the αCaMKII promoter. A mouse line will first be created in which the KIBRA gene is floxed, allowing for the deletion of the gene upon crossing with a mouse containing the Cre recombinase protein and αCaMKII promoter (Tsien *et al.*, 1996). It is hypothesized that the deletion of the KIBRA gene will be restricted to the forebrain, the memory center of the brain, and will result in decreased memory.

2. The second goal of this proposal is to examine the spatial learning and working memory of these mouse models in comparison to a control group to determine if memory has deteriorated as hypothesized.

Experimental Procedure

Previous work had showed that a SNP in the KIBRA gene is associated with memory. However, the pathway through which this association occurs is not understood. In order to verify the relationship between the KIBRA gene and episodic memory, a conditional knockout mouse will be produced to determine if deteriorated episodic memory ensues. A promoter specific to the hippocampus of the forebrain, αCaMKII, will be used. KIBRA has been found to be highly expressed in the liver and kidney, while intermediate expression was detected in the brain, lung, pancreas, and ovary (Nagase *et al,* 1998). This tissue-specific promoter will, therefore, ensure that KIBRA activity in other tissue areas is not affected; furthermore, KIBRA will only be deleted in the hippocampus and not other regions of the brain, such as the hypothalumus, which are not involved in memory. The αCaMKII promoter has been shown to initiate Cre/loxP recombination during the middle of the third postnatal week in murine models (Tsien *et al.*, 1996); this characteristic is critical, for KIBRA is thought to be involved in brain development and memory formation. The promoter will prevent inhibition of prenatal neural development and will delete the KIBRA gene only in the memory-related brain region of the hippocampus.

The sequence flanking the KIBRA gene will first be obtained from the NCBI website. A modified targeting vector containing two loxP sites, the neomycin resistance gene (neo), as well as the thymidine kinase (tk) gene, will be obtained. Via pronuclear injection of an embryonic mouse stem cell (ES cell), this vector will be used to flank the KIBRA gene with loxP, as well as insert the neomycin resistant gene (Dragatsis & Zeitlin, 2000; Erdmann *et al.,* 2007). G418 will be used to ensure the neomycin gene is transferred while ganciclovir will be added to cells with G418 resistance to ensure homologous recombination; non-homologous integration will result in cell death due to the remaining tk gene. Surviving ES cells will be injected into the inner cell mass of a fresh blastocyst and implanted into a pseudo-pregnant female (Dragatsis & Zeitlin, 2000; Erdmann *et al.,* 2007). Chimeric offspring will be crossed with wild-type mice to generate heterozygotes, which will be bred to obtain floxed KIBRA homozygotes.

Mice containing the αCaMKII-Cre transgene will be obtained from Riken Bioresource Center: Experimental Animal Division (http://www2.brc.riken.jp/lab/animal/detail.php?brc_no=RBRC00254). Crossing these mice with mice homozygous for the floxed KIBRA gene will produce offspring in which the KIBRA gene will be deleted upon activation of the αCaMKII promoter. This activation will naturally occur during the middle of the third postnatal week, ensuring the completion of neurological development (Tsien *et al.*, 1996).

To determine if deteriorated memory ensues, these mice will undergo a series of spatial learning and working memory tests, including radial-arm mazes and water maze test as described by Huentelman *et al.* (2009). Scores will be examined as the mice age to determine if a memory decline is observed as KIBRA inactivation develops. In situ hybridization and immunochemistry of brain sections will be

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performed as detailed by Johannsen *et al.* (2008) to examine KIBRA levels in various tissue sections every three weeks. Levels of the KIBRA protein and memory scores of transgenic mice will be compared to wild-type mice.

Due to the KIBRA deletion, it is expected that transgenic mice will exhibit deteriorated spatial learning and working memory. This result would confirm KIBRA's role in memory, as well as the relationship KIBRA has to the aforementioned drug hydroxyfasudil, a pharmaceutical used in wild-type mice to improve memory. Furthermore, these transgenic mice will be used as a model to identify other pharmaceuticals that improve memory impairment related to the KIBRA gene.

It is expected that KIBRA will be deleted in only the forebrain and that no deletions will occur until three weeks after birth. However, if development does not proceed normally or a lethal phenotype ensues, the tetracycline system (Tet-off) will be examined as a possible substitute to the conditional knockout described in this report. This alternative experiment, however, is beyond the scope of this proposal.

Conclusion

These experiments will determine if the deletion of the KIBRA gene in the forebrain, the memory center of the brain, results in impaired memory. This information will not only confirm KIBRA's association with memory, but will provide a murine model to elucidate the mechanism behind this relationship. Furthermore, this model could be used to identify other pharmaceuticals that improve memory impairment related to the KIBRA gene.

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