DIFFUSION TENSOR IMAGING INVESTIGATION OF KIBRA GENOTYPES

By

SINDHU PANDURANGI

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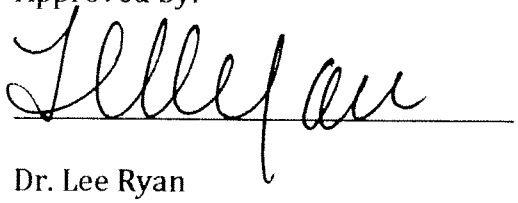

Dr. Lee Ryan
Department of Psychology

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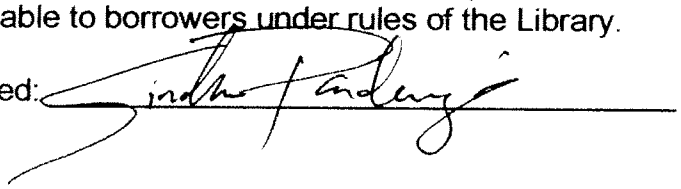
Signed: 

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Abstract

A sample of 130 adults over the age of 50 underwent diffusion tensor imaging and KIBRA genotyping. The relations between KIBRA genotype and white matter integrity were investigated through measures of Fractional Anisotropy and Apparent Diffusion Coefficient. No statistically significant findings were obtained.

Introduction

Studies in the field of neurocognitive aspects of aging are an area of psychological research that is becoming increasingly important. According to US Census Bureau data from 2004, individuals over the age of 65 will rise from 40.2 million in 2010 to 71.5 million in 2030. The aging population is growing increasingly in relation to the general population. Given this increase, understanding of the physical and cognitive changes associated with aging is imperative. The overarching research question that much of research in aging strives to answer is what are the contributing factors that lead to healthy versus pathological aging?

The specific factor considered in this study is the role of genetics in aging. In the past, studies based in genetics were restricted to using family histories and molecular biology techniques for localizing genetic mutations involved in disease expression. Recently, a new technique, referred to Genome-Wide Association Studies (GWAS), has allowed for the ability to survey an entire genome and comparing it to a representative reference population's genome for genetic variances. GWAS is essentially a population-based approach toward investigating genetic variations that may have an effect on a trait or behavior. The representative reference populations that are used in GWAS studies

were found through the International HapMap Project. In this project, populations of racially and ethnically similar individuals had their genomes screened. The Yoruba population in Nigeria, the CEPH/CEU population from Utah, and the Han population in China are all examples of representative reference populations used in GWAS. Through this process, common loci for single nucleotide polymorphisms are possible to identify for different races.

The different alleles for the gene of interest, KIBRA, were discovered using GWAS techniques. KIBRA is a protein that is expressed in both the kidney and the brain. KIBRA has been shown to be localized in the hippocampi, temporal lobes, cerebellum, and hypothalamus (Johannsen et al 2008). At the neuronal level, KIBRA has been suggested to be involved in long term potentiation and episodic memory. GWAS studies have revealed that KIBRA is located on chromosome 5, and the three allele combinations of the gene are CC, CT, and TT (Papassotiropolous et al 2006).

Perhaps the most seminal study in the investigation of KIBRA on memory and aging was conducted by Papassotiropolous et al in 2006. Two Swiss cohorts and one American cohort were tested in this study. In the first Swiss cohort, it was found that carriers of the T allele had better free recall performance after five minutes ($p = 0.00004$) and after 24 hours ($p = 0.00008$). Similar results seen in cohort 1 were seen in Swiss cohort 2, in which participants were given an episodic memory test. The U.S cohort was analyzed in an episodic memory fMRI study. This cohort showed carriers with greater right hippocampal, bilateral medial frontal gyrus, and right inferior parietal lobule activations than non-carriers. The activation was specific to episodic memory tasks and

was seen only during retrieval, not encoding (Papassotiropolous et al 2006). The over-activation seen in the carriers is consistent with the hypothesis of compensation that is commonly seen in much of aging literature. The theory of compensation essentially posits that with aging and neurocognitive decline, greater activation and bilateral activation will be seen in the brain to perform functions that would generally be localized to specific areas in a cognitively healthy, young brain (Park et al 2009). These findings suggests that the T allele KIBRA carriers may not see as significant of a decline in episodic memory with age as non-carriers.

Many other studies have replicated the findings seen in the study conducted by Papassotiropolous et al. A study conducted by Schaper et al (2008) found that T-allele carriers performed significantly better than non-carriers on the German version of the AVLT delayed free recall. A study by Almeida et al (2008) also found significantly better scores for T-allele carriers on delayed recall tests than non-carriers. In addition, Bates et al (2009) found that T-allele carriers significantly outperformed non-carriers on delayed recall tests in two separate elderly cohorts.

An earlier study conducted by the Cognition and Neuroimaging Laboratories at the University of Arizona sought to look at the cognitive effects of KIBRA as well. The goals of the study were threefold. First, since few studies had investigated KIBRA and cognitive decline in elderly adults, the study sought to replicate the effects in a larger sample size. Second, as previous studies had primarily analyzed KIBRA's association to episodic memory in small sets of 2 to 3 neuropsychological tests, the study analyzed KIBRA's effects in relation to an expanded series of episodic memory tests. Third, in

addition to episodic memory, the study investigated KIBRA's association with tests of executive function. Through this comparison, it would be possible to determine if KIBRA's effects were specific to episodic memory or also affected other aspects of cognitive function as well. We expected that KIBRA T allele carriers would outperform non-carriers on episodic memory tasks but show no relative difference to non-carriers on tests measuring executive function. Surprisingly, it was found that the effect of KIBRA was lost in our expanded sample size of 198 older adults, with no significant differences found between T allele carriers and noncarriers on any of the cognitive measures taken.

This thesis project to sought to determine if there were differential anatomical underpinnings between the genotypic groups that had not manifested as cognitive impairments. Diffusion tensor imaging allows for assessment of white matter integrity, through measures such as Fractional Anisotropy (FA) and Apparent Diffusion Coefficient (ADC). FA measures the directionality of water molecule flow, and ADC measures the average rate of diffusion of water molecules. Highly structured, healthy white matter should have a high FA value and a low ADC value. This study investigates whether FA and ADC measures differ across KIBRA genotypes, with the prediction that the T-allele carriers will have greater white matter integrity, and therefore, higher FA measures and lower ADC measures, than non-carriers.

Methods

Subjects:

A sample of 130 elderly adults were analyzed in this study. Subjects were recruited from a previous study in University of Arizona's Cognition and Neuroimaging Laboratory related to risk for Alzheimer's Disease (AD). Subjects with AD or mild cognitive impairment were not included in the data set. Participants were a part of a longitudinal study that tested neuropsychological measures every two years. Participants were asked for permission to provide a saliva sample at the second testing time to determine their KIBRA genotypes. Individuals who were not due for their second testing were phoned and asked for their saliva samples.

Within the group of 130 participants who underwent neuroimaging, 68 participants were T allele carriers for KIBRA, and 62 participants were noncarriers. Among the T allele carriers, 16 participants were males, and 52 were female. The average age of this group was 70.3 years (SD = 9.63). The average years of education in this group was 15.1 years (SD = 2.69). Within the noncarrier group, 15 participants were male, and 47 participants were female. The mean age of this subset was 71.1 years (SD = 8.92), and the average years of education was 16.2 years (SD = 2.76).

MRI Acquisition and Image Processing:

Diffusion data was collected in a previous study in our laboratory (see Ryan, Walther, Bendlin, Lue, Walker, & Glisky, 2011). Magnetic resonance images were acquired on a GE 3.0T Signa VH/I whole body echo-speed scanner equipped with an 8-channel phased array head coil (HD Signa Excite, General Electric, Milwaukee, WI). Fifty-eight axial sections of 2.6 mm thickness, no gap, covering the whole brain were acquired (TE/TR = 71ms/13000ms, matrix 96×96, FOV = 250 × 250mm2), resulting in

isotropic voxels with a resolution of 2.6mm3. Diffusion was measured in 25 directions with 2 averages (B0 = 1000s mm2, 2 NEX). Diffusion images were resampled prior to downloading at 256 × 256 using sinc interpolation. Images were realigned to remove linear eddy current distortions using the Functional Software Library (FSL) package (www.fmrib.ox.ac.uk/fsl). DTI Studio Version 2.4 (Jiang et al., 2006; https://www.dtistudio.org/) software was used to compute a diffusion tensor for each voxel that included three eigenvalues and eigenvectors. Based on these values, fractional anisotropy (FA) maps and apparent diffusion coefficient (ADC) maps were computed for each participant. Diffusion maps and T1-weighted images from each individual were then oriented parallel to the anterior-posterior commissural line and co- registered to each other using SPM2 and left in native space (Welcome Department of Cognitive Neurology, London, UK, http://www.fil.ion.ucl.ac.uk/spm/).

Region of interest (ROI) templates were created using MRIcro software (Rorden, www.mricro.com) and included fully-volumed regions of the frontal white matter, lateral parietal white matter, the centrum semiovale, the genu of the corpus callosum, the splenium of the corpus callosum, and the temporal stem white matter. ROI's for the frontal and parietal white matter, centrum semiovale, and temporal stem were created bilaterally on a representative T1 brain chosen from the sample. The frontal white matter was measured on 21 contiguous sections in the axial plane beginning two sections inferior to the anterior commissure. The ROI was placed in the center of the frontal white matter with the most posterior extent of the ROI corresponding to a plane level with the center of the genu. The centrum semiovale was measured on 11 contiguous sections in the coronal plane beginning five sections anterior to the anterior commissure and ending

five sections posterior to the anterior commissure. The ROI was placed on fully volumed white matter adjacent to the ventricles and superior to the caudate nucleus. The temporal stem white matter was measured on 23 contiguous coronal sections beginning at the section showing fully-volumed anterior commissure. The parietal white matter was measured on 12 contiguous sections in the coronal plane beginning one section posterior to fully-volumed inferior colliculi. The ROI was placed on the white matter adjacent to the ventricles and superior to the level of the lateral fissure.

Rather than using a standardized template for the corpus callosum, ROIs for the genu and splenium of the corpus callosum were drawn manually on the native space SPGRs for each participant because of the large variability in callosal shape and width across participants. The genu ROI was outlined on sagittal images beginning at the midsagittal section and continuing over the next five contiguous lateral sections in both hemispheres for a total of 10 sections. The posterior boundary was set at the most anterior point in the curve of the genu. The splenium ROI was drawn on consecutive sagittal images beginning at the midsagittal section and continuing over the next six lateral sections in both hemispheres for a total of 12 sections. The anterior boundary was set at the posterior commissure. Interrater reliability was determined for these two regions using a random sample of 10 brains. The intraclass correlations for the genu and splenium were 0.95 and 0.97, respectively, demonstrating high reliability for the procedure.

In order to ensure that diffusion values were extracted only from white matter tissue and not partially volumed gray matter, T1 images were segmented into gray matter, white matter, and cerebrospinal fluid using SPM2. We applied the segmentation procedure used

in optimized voxel based morphometry (Good et al., 2001; Gaser, http://dbm.neuro.uni-jena.de/vbm.html) which uses information about the known location of the tissue types (priors) in addition to the signal intensity of voxels obtained from actual scans. The priors were created from a random sample of 100 participants in the present study. To minimize the potential of partial voluming effects, a conservative threshold was applied to the white matter map with a white matter probability of 0.9 to create a binarized white matter mask. The binarized mask was transformed back to native space by applying the inversion of the normalization parameters for a given individual. The ROI templates described earlier were then applied to individual participant's diffusion maps and were then multiplied by the individualized white matter mask, thereby ensuring that only fully volumed white matter was included in the ROI for a given individual. This segmentation procedure had the added benefit of removing regions of white matter hyperintensity that were visible on T2-flair images. The low signal values of abnormal white matter on T1 images ensured that these regions were excluded from the white matter mask, as evident from visual comparison between the white matter mask and T2-flair images. Thus, diffusion measures were only obtained from normal-appearing white matter. Diffusion values from each ROI were averaged across all extracted voxels.

Results

A univariate analysis of variance was conducted to analyze if there was a significant difference between the two genotypes for each of the 6 regions of interest. Years of education was held as a covariate in this analysis because previous studies have suggested a protective effect of higher education on white matter integrity and cognitive

functioning in older populations (Teipel et al 2009, Ampero et al 2008, McDowell, Xi, Lindsay, and Tierney 2007, Katzman 1993).

Figure 1

Univariate Analysis of Variance for FA Measures, Years of Education as a Covariate

ROI	F	Sig.
Centrum	2.79	0.09
Frontal	0.36	0.55
Parietal	0.71	0.40
Genu	0.18	0.67
Splenium	0.84	0.36
Tempstem	0.00	0.99

Figure 2

Univariate Analysis of Variance for ADC Measures, Years of Education as a Covariate

ROI	F	Sig.
Centrum	0.51	0.48
Frontal	0.45	0.50
Parietal	0.96	0.33
Genu	0.41	0.53
Splenium	0.14	0.71
Tempstem	1.70	0.19

Since KIBRA is primarily localized to the temporal lobes, the tempstem ROI was of particular interest.

Figure 3

Mean Tempstem FA Measures Compared to Age

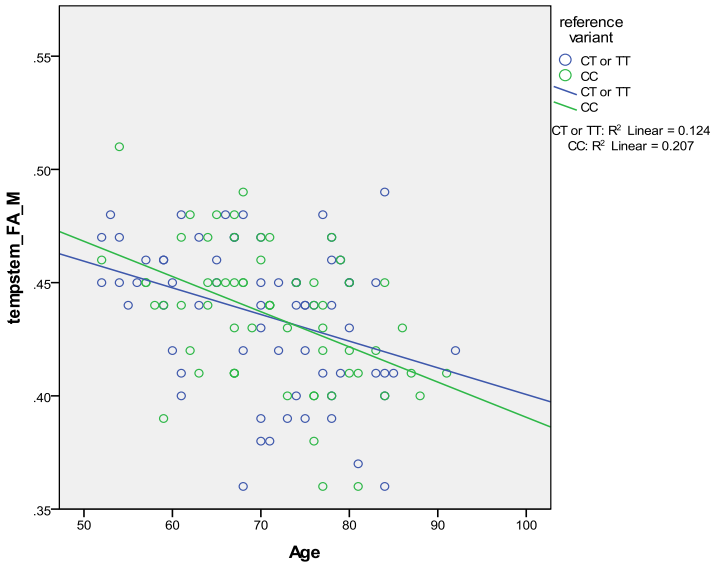
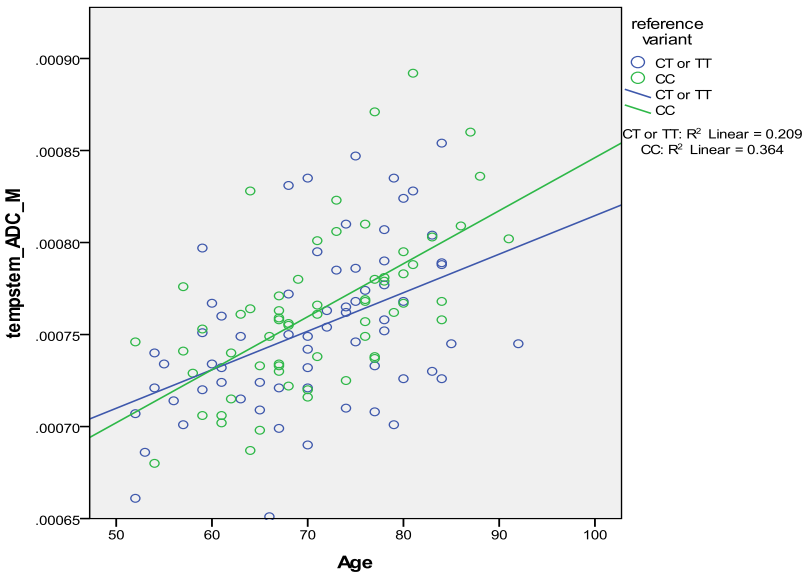


Figure 4

Mean Tempstem ADC Measures Compared to Age



Discussion and Future Directions

The univariate analysis of variance taken for both FA and ADC measures showed no significant difference between KIBRA genotypes for any of the 6 regions of interest analyzed. It should be noted that the FA measure for the centrum appears to be trending toward significance ($F(1,130) = 2.79, 0.09$); however, based on available information regarding the localization of KIBRA in the brain, there appears to be no plausible reason to heed further analysis of this result. Taken together, null findings from the cognitive study conducted earlier in Cognition and Neuroimaging Laboratories at the University of Arizona along with no significant difference found in the white matter integrity between the KIBRA genotypes in the present study, casts doubts on the effect of KIBRA in larger sample sizes.

It is possible that other genetic factors or health conditions of the participants in our sample were masking any potential effects of KIBRA. Future studies in the investigation of KIBRA could focus on segregating the carrier and noncarrier groups based on other factors aside from age and years of education. Other health and lifestyle factors, such as diabetes and hypertension, could be interacting with any potential effects of KIBRA. Studies have shown that hypertensive individuals are placed at a greater risk for developing white matter lesions ((Takami, Yamano, Okada, Sakuma & Morimoto, 2012), Dufoil et al 2001, Lee et al 2000), and studies have shown similar findings in regards to individuals with diabetes (Murray, Staff, Shenkin, Deary, Starr & Whalley, 2005, Starr, Wardlaw, Ferguson, MacLullich, Deary & Marshall, 2003)

Furthermore, KIBRA's interaction or influence under other genes that are involved with neurocognitive decline with age, such as APOE4 (Ryan, Walther, Bendlin, Lue, Walker & Glisky, 2011) could serve as yet another venue of study. Though much of human genetics remains to be explored, the fact that genes hardly act alone is rarely disputed. It is very possible that there were too many genetic variables at play in our sample of human subjects. Perhaps the most appropriate mode of studying KIBRA's effects would be through the use of an animal model, such as a transgenic mouse, which would allow for extraneous genetic factors to be controlled for. The effects of KIBRA could be studied in isolation through this approach and has the potential to lead to a better understanding of KIBRA's influence on cognitive decline with age.

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