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Association of common *KIBRA* variants with episodic memory and AD risk

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Abstract

KIBRA SNP rs17070145 was identified in a GWAS of memory performance, with some but not all follow-up studies confirming association of its T allele with enhanced memory. This allele was associated with reduced Alzheimer's disease (AD) risk in one study, which also found

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overexpression of *KIBRA* in memory-related brain regions of ADs. We genotyped rs17070145 and 14 additional SNPs in 2571 LOADs vs. 2842 controls, including African-Americans. We found significantly reduced risk for rs17070145 T allele in the older African-American subjects ($p=0.007$) and a suggestive effect in the older Caucasian series. Meta-analysis of this allele in >8000 subjects from our and published series showed a suggestive protective effect ($p=0.07$). Analysis of episodic memory in control subjects did not identify associations with rs17070145, though other SNPs showed significant associations in one series. *KIBRA* showed evidence of overexpression in the AD temporal cortex ($p=0.06$) but not cerebellum. These results suggest a modest role for *KIBRA* as a cognition and AD risk gene, and also highlight the multifactorial complexity of its genetic associations.

Keywords

Alzheimer's disease; Association studies in genetics; Case control studies

INTRODUCTION

Papassotiropoulos and colleagues (Papassotiropoulos, et al., 2006) identified an association between human episodic memory and a common *KIBRA* (Kidney and brain expressed protein) SNP (single nucleotide polymorphism) (rs17070145) among 341 healthy, young Swiss adults (median age=22), with replication in two additional healthy cohorts from Switzerland ($n=424$, median age=21) and USA ($n=256$, median age=55). Compared to non-carriers, subjects with the rs17070145 T allele showed better delayed recall across a variety of episodic memory tasks and less hippocampal activation on functional magnetic resonance imaging during an episodic memory task. Follow-up studies have replicated the original association with delayed recall (Almeida, et al., 2008, Preuschhof, et al., 2010, Schaper, et al., 2008, Vassos, et al., 2010), found significant association but in the opposite direction (Nacmias, et al., 2008), found significant association but only when controlling for initial learning (Bates, et al., 2009) or found no significant association (Need, et al., 2008). Thus, although some of these studies suggest a plausible association between *KIBRA* and episodic memory, the aggregate results remain difficult to interpret given the differences in sample size, country of origin, age at evaluation, and cognitive tests examined (Schneider, et al., 2010).

In addition, two studies have assessed the role of rs17070145 variant in AD risk. Corneveaux and colleagues compared 1629 AD cases to 936 controls from 6 different sources in the USA, Germany, Norway, and Netherlands and found significantly decreased AD risk in rs17070145 T allele carriers (Corneveaux, et al., 2008). In contrast, Rodriguez-Rodriguez and colleagues did not find evidence of AD risk association in their 391 AD versus 428 control subjects from Northern Spain (Rodriguez-Rodriguez, et al., 2007). When stratified by age, the authors found a significantly risky association among adults older than 86 years, but in the opposite direction (i.e., rs17070145 T allele associated with increased risk of AD).

KIBRA interacts with a multitude of proteins involved in synaptic function, cell polarity, vesicular transport and neuronal plasticity (Schneider, et al., 2010). *KIBRA* is expressed in memory-related structures of the brain (Johannsen, et al., 2008) and has increased expression in laser-capture microdissected neurons from the hippocampus, middle temporal gyrus, and posterior cingulate (but not the pathologically spared primary visual cortex) of AD cases in comparison to controls (Corneveaux, et al., 2010). Thus far, the available genetic and functional evidence supports *KIBRA* as an interesting candidate gene for cognition and

LOAD risk, but given the conflicting reports further evidence from independent studies appears warranted.

To investigate the role of *KIBRA* in LOAD risk, we genotyped rs17070145 along with 14 additional SNPs in >5400 subjects (2571 LOAD vs. 2842 elderly controls), including 371 African-American subjects (119 LOAD vs. 252 controls). We also evaluated rs17070145 in a meta-analysis combining our data and all published series, collectively composed of >8000 subjects. Furthermore, we evaluated the role of *KIBRA* variants and episodic memory in >2000 of our elderly control subjects. Finally, we measured *KIBRA* mRNA expression levels in the temporal cortex and cerebellum from post-mortem AD and non-AD brains. To our knowledge this represents the largest case-control study to date assessing *KIBRA* variants for their role in LOAD risk and cognition. Our results provide additional support for *KIBRA*'s role in cognition and AD risk, but also highlight the multifactorial complexity of these genetic associations.

MATERIALS AND METHODS

Patient samples

We collectively analyzed 2571 LOAD subjects and 2842 elderly controls, where 119 LOADs and 252 controls were African-American and the remaining subjects were Caucasian-Americans. The Caucasian-American series were as follows: Clinically-diagnosed series from Mayo Clinic Jacksonville (JS_OLD and JS_YOUNG), Mayo Clinic Rochester (RS_OLD and RS_YOUNG), National Cell Repository for AD (NCRAD_OLD and NCRAD_YOUNG), and autopsy-confirmed series maintained at the Brain Bank at Mayo Clinic Jacksonville (AUT_OLD and AUT_YOUNG). The African-American (AA_OLD and AA_YOUNG) series were collected at Mayo Clinic Jacksonville. All Caucasian-American series were grouped using age of diagnosis (for clinical LOADs), or age at evaluation (for clinical controls) or death (for autopsy LOADs and controls) of 60-80 years (_YOUNG) and >80 years (_OLD). Because African-American subjects were younger at the time of diagnosis/evaluation than their Caucasian-American counterparts, their median age at the time of diagnosis/evaluation was chosen to designate the AA_OLD (>age 74) and AA_YOUNG (60-74) series. The details of these series are available in Supplementary Table 1.

All subjects from the AA, JS and RS series were diagnosed by a Mayo Clinic neurologist. The neurologist confirmed a Clinical Dementia Rating (CDR) score of 0 for all subjects enrolled as controls; cases had diagnoses of possible or probable AD made according to NINCDS-ADRDA criteria (McKhann G, et al., 1984). In the autopsy-confirmed series all brains were evaluated by the neuropathologist (D.W.D.), where diagnosis of definite AD was also made according to NINCDS-ADRDA criteria. None of the AUT control subjects had diagnosis of AD, but many had brain pathology unrelated to AD and pathological diagnoses that included vascular dementia, frontotemporal dementia, dementia with Lewy bodies, multi-system atrophy, amyotrophic lateral sclerosis, and progressive supranuclear palsy. The NCRAD LOAD and control subjects were also diagnosed by NINCDS-ADRDA and CDR of 0, respectively. One AD case from each of the 715 late-onset NCRAD families and unrelated NCRAD controls was analyzed, as previously described (Zou, et al., 2010).

This study was approved by the appropriate institutional review board and appropriate informed consent was obtained from all participants.

SNP genotyping and data quality control (QC)

15 SNPs within or flanking *KIBRA* (aka *WWCI*) were genotyped. These SNPs were chosen because they were HapMap tagging SNPs (Frazer, et al., 2007), were within regions that

have >70% interspecies conservation (human and mouse) in 100 bp sliding windows, were previously published variants (Papassotiropoulos, et al., 2006), or a combination of these factors (Supplementary Table 2). All SNPs were genotyped using the Sequenom platform (Oeth P, et al., 2006). All genotype data was assessed for QC using PLINK (Purcell, et al., 2007). None of the SNPs were in violation of Hardy-Weinberg equilibrium in the controls at $p < 0.01$ in any of the series or all series combined. All SNPs had a genotyping success rate of $\geq 90\%$ in each series and all series combined. All SNPs were tested for association with AD risk (Supplementary Table 3) and episodic memory phenotypes (Supplementary Tables 4 and 5).

Episodic Memory Phenotypes

The Auditory Verbal Learning Test (AVLT) (Rey, 1964) consists of 15 semantically-unrelated nouns presented orally over 5 consecutive learning trials. After an interference trial in which 15 novel nouns are presented, subjects are asked to freely recall as many words from the original list as possible. After a 30-minute delay, subjects are asked again to freely recall as many of the original words as possible (delayed recall trial). To remain consistent with the original *KIBRA* report (Papassotiropoulos, et al., 2006), we utilized the delayed recall score (AVD) as one of our episodic memory phenotypes. The AVLT at the time of their last evaluation was available for 2174 Caucasian and 224 African-American subjects who were clinically normal at the time of their last evaluation (Supplementary Table 4).

We also investigated the association between *KIBRA* and the Logical Memory subtest from the Wechsler Memory Scale-Revised (Wechsler, 1987). Logical Memory consists of two prose stories followed by immediate and 30-minute delayed recall trials. We utilized the Logical Memory delayed recall score (MRLMD) as our second episodic memory phenotype. Logical Memory at the time of their last evaluation was available for 1973 Caucasian and 215 African-American subjects who were clinically normal at the time of their last evaluation (Supplementary Table 5).

Statistical Analyses

Each SNP was assessed individually for association with LOAD by multivariate logistic regression analysis using an allelic dosage model, adjusted for *APOE4* dosage (0, 1, 2), age at diagnosis/evaluation/death, and sex. Association between the same *KIBRA* SNPs and AVLT delayed recall scores (AVD) or delayed Logical Memory scores (MRLMD) at last evaluation were tested in additive multivariate linear regression models adjusted for *APOE4* dosage, age at evaluation, and sex. All analyses were done in PLINK (Purcell, et al., 2007).

To perform a meta-analysis of our and all published series for *KIBRA* SNP rs17070145 allelic associations, allelic counts were calculated using the reported allelic frequency and sample size information from the literature (Corneveaux, et al., 2008; Rodriguez-Rodriguez, et al., 2007) or AlzGene website (Bertram, et al., 2007). Breslow-Day test for non-compatibility was used to test for series heterogeneity. Test statistics are reported for each series and pooled test statistics are reported using the random effects model (DerSimonian-Laird). Analysis of linkage disequilibrium (LD) was done in HaploView (Barrett, et al., 2005) using the solid spine of LD method for all 15 SNPs. Results that are nominally significant at $p < 0.05$ and marginally significant at $p < 0.25$ (Hosmer DW and Lemeshow S, 2000) are highlighted in this study.

Gene Expression

Many subjects from the AUT series had temporal cortex and/or cerebellar tissue that underwent gene expression measurements. TaqMan® Arrays (Jiang, et al., 2006; Steg, et al.,

2006) were used for the gene expression studies. Inventoried TaqMan® Assays were used to measure expression of *KIBRA* (Hs00392086_m1), as well as the three control genes (*GAPDH*=Hs99999905_m1; *TFRC*=Hs99999911_m1; *HPRT1*=Hs99999909_m1). The TaqMan assay used to measure *KIBRA* gene expression levels can identify all three known mRNA transcripts for this gene. RNAqueous Kit® from AB (Applied Biosystems) was used to extract total RNA from temporal cortex. Total RNA was extracted from cerebellar samples using the AB RNA Chemistry for Tissue Samples and an AB PRISM™ 6100 Nucleic Acid PrepStation according to the manufacturer's instructions. The quantity and quality of the RNA samples were determined by the Agilent 2100 Bioanalyzer using the Agilent RNA 6000 Nano Chip. The High-Capacity cDNA Archive kit from AB was used to reverse transcribe cDNA from mRNA. The measurements were submitted to QC and outliers excluded as previously published (Zou, et al., 2010).

Each sample was analyzed in triplicate with 3-4 ng starting cDNA amount. Following thermal cycling, the plates were read on an AB Prism 7900HT sequence detection system. The SDS 2.2 software package from Applied Biosystems was used to obtain the raw data. The variable C_t within the raw data file indicates the PCR cycle number at which the amount of amplified gene target reaches a fixed threshold. The variable ΔC_t denotes the difference between the averaged C_t values for the target gene replicates and that for the geometric mean of the reference gene replicates (i.e. *GAPDH*, *TFRC*, *HPRT1*). To analyze relative expression levels, $2^{-\Delta C_t}$ values were used. The effects of diagnosis on the relative expression of *KIBRA* were assessed separately for cerebellar and temporal cortex measurements using diagnosis (AD=1, non-AD=0), age at death, sex, *APOE4* dosage, and RNA integrity numbers (from Agilent) as covariates, in a multivariate linear regression paradigm.

We also assessed the association of each of the 15 SNPs with *KIBRA* gene expression levels in the cerebellum or temporal cortex of the autopsy subjects using the ΔC_t values as the quantitative phenotype; and the additive SNP genotype and all the covariates above in a multivariate linear regression model within PLINK (Purcell, et al., 2007).

RESULTS

AD Risk Association of *KIBRA* SNP rs17070145

Using logistic regression analysis and controlling for age at diagnosis/evaluation/death, *APOE4* dosage, and sex, we tested for association of the *KIBRA* SNP rs17070145 with AD risk in an additive model, in 2452 Caucasian-Americans with LOAD vs. 2590 controls and 119 African-Americans with LOAD vs. 252 controls. *KIBRA* SNP rs17070145 T allele is the minor allele in Caucasians, but is the major allele in the African-Americans (Supplementary Table 2). We found a significantly reduced risk of LOAD in our older African-American (AA_OLD) series (Table 1a) associated with the T allele (OR=0.50, 95%CI=0.30-0.83, $p=0.007$). Similarly, two out of four of our older Caucasian-American series showed marginally reduced LOAD risk association with the T allele, where JS_OLD series had OR=0.78, 95%CI=0.58-1.04 ($p=0.09$) and RS_OLD series had OR=0.85, 95%CI=0.68-1.07 ($p=0.17$). A third older Caucasian series, NCRAD_OLD, had a protective OR estimate (OR=0.90, 95%CI=0.58-1.40) and the AUT_OLD series had a risky estimate (OR=1.08, 95%CI=0.74-1.56), though neither was significant. Among the younger series, AUT_YOUNG had marginal association of the T allele in the opposite and risky direction (OR=1.21, 95%CI=0.91-1.62, $p=0.19$), and no other younger series showed marginal ($p<0.25$) or significant ($p<0.05$) association. When all older Caucasian series were assessed jointly, there was a marginal trend towards a protective effect (OR=0.88, 95%CI=0.77-1.02, $p=0.08$) for the T allele. There was no significant association in the Caucasian series when all series from both age groups were jointly assessed.

Meta-analysis of AD Risk Association with *KIBRA* SNP rs17070145 T allele

A meta-analysis of *KIBRA* SNP rs17070145 T allelic association with AD risk in all of Mayo Clinic series, either including (Mayo, 2520 LOAD vs. 2753 controls) or excluding African-American subjects (Mayo_Caucasian, 2403 LOAD vs. 2511 controls), yielded protective OR estimates that were not significant (Table 1b). When the Mayo Clinic series were assessed jointly with data from published series (Corneveaux, et al., 2008, Li, et al., 2008, Rodriguez-Rodriguez, et al., 2007), the meta-analysis p-value for association showed marginal significance ($p=0.07$) with a protective OR estimate for the T allele ($OR=0.94$), regardless of whether the African-American series was included (Figure 1).

AD Risk Association of 15 SNPs Within and Flanking *KIBRA*

In addition to rs17070145, we assessed 14 SNPs within and flanking *KIBRA* for association with AD risk (Supplementary Table S3). As expected, rs1477306, which is in the same haplotype block as rs17070145 (Supplementary Figure 1), showed significant association with AD risk in the older African-American (AA_OLD), and marginal association in the JS_OLD and RS_OLD series, with the A allele being protective. Besides these two SNPs with marginal significance in the combined older Caucasian series (ALL-Caucasian_OLD; Supplementary Table S3), rs1030182 was the only other SNP with nominal significance in the combined older Caucasian series, with the A allele having a risky OR estimate ($OR=1.15$, $95\%CI=1.01-1.31$, $p=0.04$). This SNP was also significantly risky ($OR=1.61$, $95\%CI=1.02-2.55$, $p=0.04$) in the AA_OLD series. No other SNPs showed marginal or significant association of consistent effects in three or more of the series tested. No SNPs were significant at $p<0.05$ in any of the younger series tested. It should be noted that with a strict Bonferroni correction applied for 15 SNPs, no associations achieved corrected statistical significance at $p<0.05$, but SNPs rs17070145, rs1477306 and rs7700355 retained marginal significance of $p\sim 0.11-0.14$ in the AA_OLD series.

Episodic Memory Association with 15 SNPs Within and Flanking *KIBRA*

KIBRA SNP rs17070145, which had a significant association with AVLT delayed recall in the original study (Papassotiropoulos, et al., 2006), did not show significant association with delayed recall using either the AVLT (AVD) or Logical Memory (MRLMD) tests, in any of the series examined (Supplementary Tables S4 and S5). When considering the remaining 14 SNPs within and flanking *KIBRA*, the majority of associations with episodic memory were obtained in the older JS_OLD series. Specifically, 6 SNPs showed nominally significant association with AVD and 5 SNPs with MRLMD at $p<0.05$. Three of the AVD (rs11750709, rs1422422, rs4976592) and one MRLMD (rs4976592) associations in the JS_OLD series remained significant after Bonferroni correction for 15 SNPs tested. The rs4976592 T allele, which was associated with worse AVD and MRLMD scores in the JS_OLD series, had a marginal trend in the same direction for both phenotypes in the AA_OLD series. Despite these findings, SNPs with significant episodic memory associations did not show significant AD risk associations and vice versa.

KIBRA Expression levels in AD and non-AD brains

We assessed *KIBRA* expression levels in mRNA extracted from homogenized temporal cortex of LOAD ($n=84$) and non-AD subjects ($n=60$). We also investigated cerebellar *KIBRA* levels in LOAD ($n=177$) and non-AD ($n=197$) subjects. After controlling for age at death, sex, *APOE4* dosage, and RNA integrity numbers (from Agilent®), we found marginally increased *KIBRA* expression in LOAD temporal cortex ($p=0.06$) compared to non-ADs (Supplementary Table S6 and Supplementary Figure S2). There was no difference in the *KIBRA* expression levels in the cerebella of LOAD vs. non-AD subjects. We also tested for association of the 15 SNPs with either the cerebellar or temporal cortex *KIBRA*

expression levels (Supplementary Table S7) and did not find any SNPs that show significant association at nominal $p < 0.05$.

DISCUSSION

Since the initial publication of a cognitive GWAS demonstrating an association between *KIBRA* SNP rs17070145 and episodic memory (Papassotiropoulos, et al., 2006), there has been interest in replicating and better understanding the nature of the relationship of *KIBRA* with cognition and AD risk. Early follow-up studies on the cognitive associations were promising (Almeida, et al., 2008, Preuschhof, et al., 2010, Schaper, et al., 2008, Vassos, et al., 2010), but a number of subsequent reports have either failed to demonstrate the association (Need, et al., 2008), or showed association in the opposite direction (Nacmias, et al., 2008). Two studies have evaluated the association between *KIBRA* rs17070145 T allele with AD, with one study finding a protective effect (Corneveaux, et al., 2008) and the other no association in their complete series and a risky effect in their oldest age group (Rodriguez-Rodriguez, et al., 2007). In light of these discrepancies, we sought to investigate the association between episodic memory and LOAD risk with rs17070145 and 14 additional SNPs within and flanking *KIBRA* in our series of 2403 AD cases and 2511 controls.

To our knowledge, our study represents the largest LOAD case-control study to date, and about 1.4 times greater in size than all published series combined. It is also the first study to evaluate *KIBRA* for cognition and AD risk in a different ethnic group, African-Americans. Despite a slightly protective risk estimate ($OR = 0.97$), our combined series did not show significant LOAD risk association with *KIBRA* rs17070145 T allele. When each case-control series was individually assessed, older African-Americans had a significant, protective association with the rs17070145 T allele, consistent with the Corneveaux et al. study (Corneveaux, et al., 2010). Two of our older Caucasian series (JS_OLD and RS_OLD) also had protective trends for this allele, as did the combined older Caucasian series, though they did not achieve nominal significance. This marginally protective effect for the T allele was further demonstrated in a meta-analysis of all of our series together with the published data on rs17070145. Finally, *KIBRA* message was found to be overexpressed ($p = 0.06$) in the temporal cortex, but not in the pathologically-spared cerebellum ($p = 0.91$), of AD subjects compared with controls, consistent in trend with a previous report (Corneveaux, et al., 2010). Our expression assay is designed to measure all three known isoforms of *KIBRA*. Therefore if there are isoform-specific differences in *KIBRA* expression in the brains of AD vs. non-AD subjects, this expression assay will not be able to detect these. Thus, it remains possible that, although we found only a trend for *KIBRA* overexpression in the temporal cortex of AD subjects, bigger differences could potentially be identified by utilizing isoform-specific assays. Although, we did not identify any associations between the 15 SNPs and *KIBRA* expression levels, there may be other *KIBRA* variants that influence its brain expression via influencing differential splicing or transcription factor binding.

These AD association findings for *KIBRA* rs17070145 SNP raise several intriguing observations. First, an age effect is evident in both our African-American and Caucasian series. Older subjects at the time of AD diagnosis/evaluation show protective trends for the rs17070145 T allele, whereas our younger subjects show no evidence of this association (except for a trend toward the opposite risky effect in our young autopsy series). A prior study also found a significant association between rs17070145 and LOAD among older adults, although the effect was in the opposite risk direction (Rodriguez-Rodriguez, et al., 2007). Although the older and younger series were not statistically different based on their overlapping 95% CIs and an insignificant Breslow-Day p value, it would be interesting to

re-evaluate all existing published series by their ages of onset to determine the influence of age AD risk association in the largest possible dataset.

Second, we show a nominally significant association between the *KIBRA* rs17070145 T allele and LOAD risk among African American adults. This effect is in the same, protective direction as that shown in the largest published Caucasian AD association study (Corneveaux, et al., 2010), as well as what may be expected by *KIBRA*'s memory-enhancing effect in cognitive studies (Papassotiropoulos, et al., 2006; Preuschhof, et al., 2010; Schaper, et al., 2008; Vassos, et al., 2010). While preliminary, this finding suggests that the effects of this gene on AD risk may generalize to different ethnic/racial groups. Given the relatively small sample size of this group this association in African-Americans needs replication by others.

Third, the marginal significance ($p=0.07$) for the rs17070145 T allele obtained in our meta-analysis of 4436 AD cases and 4334 controls likely reflects the lack of power to detect the relatively small effect conferred by this variant. Indeed, even with the current aggregate sample of nearly 9000 subjects in our meta-analysis, there is ~50% power to detect an association at $\alpha=0.05$, given the minor allele frequencies observed in the cases and controls. Furthermore, if rs17070145 is not the functional *KIBRA* polymorphism but a tagging SNP for the functional variant, then different underlying LD patterns in the different series could lead to additional heterogeneity and lack of a discernable association signal in some series, whereas others may show an association signal. Different gene-gene, gene-environment influences in different series, and presence of multiple underlying functional variants within the same gene are additional sources for potential false-negative associations.

We did not observe a significant association for the *KIBRA* rs17070145 SNP with episodic memory scores, although several other SNPs showed Bonferroni-corrected association at $p<0.05$ in one of our series (JS_OLD). The lack of a clear association between rs17070145 and our two episodic memory phenotypes stands in contrast to the original findings from Papassotiropoulos and colleagues (Papassotiropoulos, et al., 2006). It should be noted that although there are reports of both AD risk and cognitive associations with the *KIBRA* rs17070145 SNP, to our knowledge positive associations for both phenotypes in the same series has not been reported. There could be several explanations for the lack of episodic memory associations with rs17070145 in our series: 1) *KIBRA* rs17070145 SNP may influence episodic memory and AD risk differentially depending on age (i.e., a stronger effect on memory early on, but on AD risk later in life). 2) The effect of this SNP on episodic memory may be influenced by factors that have not been taken into account in this study. For example, in a recent study that also employed both the AVLT and Logical Memory tests, the authors failed to find a direct association with delayed recall scores, although they were able to demonstrate an association between AVLT delayed recall and rs17070145 after controlling for initial learning in multiple regression analyses (Bates, et al., 2009). 3) Different *KIBRA* variants may influence memory and AD risk. 4) Our memory association results for rs17070145 may represent false negatives due to the modest effect conferred by *KIBRA* and lack of sufficient statistical power. 5) It remains possible that some of the positive associations reported by other investigators may represent false positives. Investigating both AD risk and cognition concurrently in additional series will be necessary to explore these possibilities.

There are a number of significant strengths to our investigation. First, our case-control series represents the largest *KIBRA* association study reported thus far. Second, we present data on a cohort of African American adults, and analyze their association to LOAD and episodic memory uniquely with appropriate adjustments (for instance, taking into account their younger age when compared to Caucasian-Americans). Third, we did not limit our study to

a single SNP (rs17070145) but considered additional SNPs that may bear on the relationship between *KIBRA* and LOAD or episodic memory. Fourth, we pursued meta-analysis of the rs17070145 T allelic association using our entire series and the available published data to maximize the statistical power to find effects on this SNP. Fifth, we investigated brain expression levels of *KIBRA* similar to one other study (Corneveaux, et al., 2010).

In summary, genetic data from a subset of our series provides suggestive evidence for a role of *KIBRA* SNPs in AD risk and memory, although significant questions remain. *KIBRA* appears to confer at most a very modest effect in AD risk and memory, and even sample sizes in excess of 9000 subjects may be underpowered to detect this effect. Thus, clearly larger studies in both Caucasians and other ethnic groups are needed for stronger evidence in support of *KIBRA*'s role in LOAD and memory. Moreover, targeted re-sequencing efforts to identify functional variants that can subsequently be tested in both functional paradigms and association studies will provide definitive answers to some of the questions raised by this and other studies. Use of multiple phenotypes, including broad assessment across cognitive domains including and beyond episodic memory, AD risk, and gene expression levels concurrently in the same series may bring additional understanding to the genetic complexity underlying *KIBRA*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

AD Risk association of KIBRA SNP rs17070145

a) 10 Mayo Clinic series assessed by logistic regression analysis and b) Combined Mayo Clinic and published series assessed by meta-analysis.

a.	AD Risk - Logistic Regression				
	OR	L95	U95	P	
Mayo_AA_OLD	0.50	0.30	0.83	0.007	
Mayo_JS_OLD	0.78	0.58	1.04	0.09	
Mayo_RS_OLD	0.85	0.68	1.07	0.17	
Mayo_AUT_OLD	1.08	0.74	1.56	NS	
Mayo_NCRAD_OLD	0.90	0.58	1.40	NS	
Mayo_AA_YOUNG	1.20	0.66	2.18	NS	
Mayo_JS_YOUNG	1.02	0.79	1.32	NS	
Mayo_RS_YOUNG	1.03	0.82	1.31	NS	
Mayo_AUT_YOUNG	1.21	0.91	1.62	0.19	
Mayo_NCRAD_YOUNG	0.87	0.60	1.26	NS	
Mayo_Caucasian_OLD	0.88	0.77	1.02	0.08	
Mayo_Caucasian_YOUNG	1.06	0.93	1.20	NS	

Series	AD				CON				AD Risk - Meta-Analysis						
	CC	CT	TT	CC	CT	TT	CC	TT	AD-N	AD-Freq (T)	CON-N	CON-Freq (T)	OR	L95	U95
	Meta-Analysis P								AD Risk - Individual Series						
Mayo	1124	1112	284	1183	1193	377	1183	1193	2520	33%	2753	35%	0.96	0.87	1.06
Mayo_Caucasian	1108	1052	243	1148	1083	280	1148	1083	2403	32%	2511	33%	0.97	0.88	1.06
Mayo+Published	2024	1957	455	1894	1894	546	1894	1894	4436	32%	4334	34%	0.94	0.87	1.01
Mayo+Published_Caucasian	2008	1897	414	1859	1784	449	1859	1784	4319	32%	4092	33%	0.94	0.88	1.01
	AD				CON										
	CC	CT	TT	CC	CT	TT	CC	CT	AD-N	AD-Freq (T)	CON-N	CON-Freq (T)	OR	L95	U95
Mayo_AA_OLD	9	44	22	10	41	53	10	41	75	59%	104	70%	0.62	0.39	0.98
Mayo_JS_OLD	115	90	24	112	104	32	112	104	229	30%	248	34%	0.84	0.63	1.12
Mayo_RS_OLD	120	124	27	259	279	72	259	279	271	33%	610	35%	0.92	0.74	1.15
Mayo_AUT_OLD	145	141	32	52	43	8	52	43	318	32%	103	29%	1.19	0.83	1.71

Series	AD				CON				AD Risk - Meta-Analysis						Meta-Analysis P	
	CC	CT	TT		CC	CT	TT		AD-N	AD-Freq (T)	CON-N	CON-Freq (T)	OR	L95	U95	
Mayo_NCRAD_OLD	69	68	17	33	44	9			154	33%	86	36%	0.88	0.58	1.33	
Mayo_AA_YOUNG	7	16	19	25	69	44			42	64%	138	57%	1.36	0.80	2.35	
Mayo_JS_YOUNG	166	152	39	158	147	35			357	32%	340	32%	1.01	0.80	1.28	
Mayo_RS_YOUNG	125	121	23	362	303	82			269	31%	747	31%	0.99	0.79	1.23	
Mayo_AUT_YOUNG	111	119	29	122	104	28			259	34%	254	31%	1.13	0.86	1.48	
Mayo_NCRAD_YOUNG	257	237	52	50	59	14			546	31%	123	35%	0.83	0.61	1.12	
Corneveaux-PM US	232	176	49	103	110	32			457	30%	245	36%	0.78	0.61	0.99	
Corneveaux-AM Norway	65	63	15	32	54	11			143	33%	97	39%	0.75	0.50	1.11	
Corneveaux-AM German	88	117	14	44	58	11			219	33%	113	35%	0.90	0.64	1.29	
Corneveaux-AM Netherlands	8	7	1	8	4	0			16	28%	12	17%	1.96	0.45	9.95	
Li et al (AlzGene)	324	305	61	318	291	77			690	31%	686	32%	0.93	0.79	1.10	
Rodriguez et al.	183	177	31	206	184	38			391	31%	428	30%	1.01	0.81	1.25	

OR= odds ratio, L95 = lower 95% confidence interval (CI), U95 = upper 95% CI, P= p value of association. P values > 0.25 are depicted as NS = not significant. Associations that have P ≤ 0.25 are highlighted in pink if OR is risky (> 1.0) and in green if OR is protective (<1.0). Logistic regression analysis was performed, assuming an additive model and using age of onset/diagnosis/death, gender and ApoE 4 dosage as covariates. Meta-analysis includes Mayo Clinic series and all published series from Corneveaux et al.(Corneveaux, et al., 2008), Li et al.(Li, et al., 2008) (frequencies obtained from AlzGene), and Rodriguez-Rodriguez et al.(Rodriguez-Rodriguez, et al., 2007). Meta-analysis was done using a random effects model and including all series from Mayo Clinic (Mayo), after excluding the Mayo African-American series (Mayo_Caucasian), Mayo+Published series with or without the Mayo African-American series (Mayo+Published_Caucasian). AD-N = number of subjects with AD, CON-N = number of control subjects, AD-Freq (T) = Frequency of the T allele in ADs and controls (CON-Freq (T)). The number of AD and control subjects with each of the three rs17070145 genotypes are shown in Table 1b for each series and the combined series.