Genetic association between KIBRA polymorphism and Alzheimer's disease with in a Japanese population

Eri Kawai[a], Nobuto Shibata[a]*, Tomoyuki Nagata[b,c], Shunichiro Shinagawa[b], Kenji Tgai[b], Tohru Ohnuma[a], Hiromi Shimazaki[a], Aiko Toda[a], Koji Kasanuki[a], Toshiki Takayama[a], Ayako Suzuki [a], Kazuhiko Nakayama[b], Hisashi Yamada[c], Heii Arai[a]

[a] Department of Psychiatry, Juntendo University School of Medicine, Tokyo, Japan

[b] Department of Psychiatry, Jikei University School of Medicine, Tokyo, Japan

[c] Division of Molecular Genetics, Institute of DNA Medicine, Jikei University School of Medicine, Tokyo, Japan

*_Correspondence to_: Nobuto Shibata MD, PhD

Department of Psychiatry, Juntendo University School of Medicine

2-1-1 Hongo, Bunkyo-ku, Tokyo, 113-8421 Japan

TEL/FAX: (+81)-3-5802-1071

E-mail: nshibata@juntendo.ac.jp

Keywords: KIBRA; apolipoprotein E; polymorphism; alcohol; Alzheimer's disease; single photon emission computed tomography

Running head: KIBRA polymorphism and Alzheimer's disease

Word count: 3222

Tables: 5

Figures: 1

Abstract

KIBRA plays an important role in synaptic plasticity in human hippocampus related to cognitive function. Functional studies suggest that KIBRA is a potential candidate gene for memory and Alzheimer's disease (AD) risk. A single nucleotide polymorphism (SNP), Rs17070145 C allele affects the onset of AD in an age-dependent manner comparing with T/T genotypes and is also associated with risk of substance abuse and relapse. The aim of this case-control study was to investigate whether the rs17070145 polymorphism affected the onset of AD in an age-dependent manner in a Japanese population. We analysed KIBRA and APOE genotypes in 237 young AD cases, 154 age-matched control cases and 160 old AD cases. The analyses were performed by stratifying alcohol consumption and the APOE status. We used single photon emission computed tomography (SPECT) to analyse patients with AD with the rs17070145 polymorphism. The genotypic and allelic frequencies of the young AD group differed significantly from those of control and old AD groups. There was a significant association among high alcohol consumption (HAC-AD group) and the genotypic and allelic frequencies of the rs17070145 polymorphism. Logistic regression analyses demonstrate synergism between the APOE genotype and the rs17070145 C allele to increase the risk of AD in the young group; this was confirmed in the HAC-AD group. The SPECT study revealed hyperperfusion in the C allele carrier group was detected in the right inferior frontal gyrus compared with the T/T group. KIBRA rs17070145 affects specific phenotypes of patients with AD.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease. Genetic, metabolic and environmental factors play an important role in the pathophysiology of AD (Reitz and Mayeux 2014). Apolipoprotein E (APOE) is a genetic risk factor for sporadic AD (Brouwers et al. 2008). Lifestyles and lifestyle diseases such as hypertension, hyperlipidemia and diabetes mellitus affect the onset or clinical course of AD (Frisardi et al. 2010). Ethanol and its metabolite acetaldehyde are directly neurotoxic, and alcohol intake may affect the development of AD (Ohta and Ohsawa 2006). There may be potential associations among AD, alcohol abuse and certain genetic factors.

KIBRA is highly expressed in the human brain and plays an important role in synaptic plasticity (Duning et al. 2013; Schneider et al. 2010). Genome-wide analyses show that rs17070145, a frequently occurring single-nucleotide polymorphism (SNP) of the KIBRA gene, is significantly associated with memory performance in cognitively normal controls (Papassotiropoulos et al. 2006). Similar studies on the rs17070145 polymorphism that include Japanese cohorts have been performed (Almeida et al. 2008; Bates et al. 2009; Milnik et al. 2012; Preuschhof et al. 2010). A previous study reported significant associations between the rs17070145 polymorphism and verbal memory, attention/concentration and delayed recall performance (Yasuda et al. 2010). Although significant associations of elderly German subjects were reported (Schaper et al. 2008), analysis of other cohorts of European populations did not support previous findings (Need et al. 2008; Sedille-Mostafaie et al. 2012). The T allele of the polymorphism may increase the risk for late-onset AD (Burgess et al. 2011; Corneveaux et al. 2010). Another study was unable to repeat these positive results (Hayashi et al. 2010). Thus, the influence of the polymorphism on the onset of AD is discrepancy in findings. A meta-analysis including several populations showed protective effect of T allele and suggested a modest role for KIBRA as a cognition and AD risk gene

(Burgess et al. 2011).

The KIBRA polymorphism has been analyzed in other neuropsychiatric diseases (Vassos et al. 2010; Vyas et al. 2014). Evidence suggests an association between KIBRA gene polymorphism and cognitive function in schizophrenia (Vyas et al. 2014), and post-traumatic stress disorder (Wilker et al. 2013). Previous findings also suggest that rs17070145 is a putative risk factor for substance abuse and relapse (Bauer et al. 2012) and that KIBRA SNPs are related to cognitive flexibility (Zhang et al. 2009). These findings suggest a potential association between alcohol dependency and KIBRA polymorphisms. A functional magnetic resonance imaging (fMRI) study shows that the KIBRA polymorphism affects hippocampal activations during memory retrieval (Papassotiropoulos et al. 2006). They suggest non-carriers of the T allele showed significantly increased brain activations compared with those of T allele carriers in the medial temporal lobe. It means that non-carriers of the T allele need more activation to reach the same level of retrieval performance as T allele carriers. Another fMRI study on normal healthy subjects indicates that T-allele carriers have a larger hippocampal volume compared with non-carriers (Kauppi et al. 2011). However, to our knowledge, there are no previous neuroimaging studies investigating the influence of KIBRA gene polymorphism in patients with AD.

The present study aimed to clarify the associations between the KIBRA rs17070145 polymorphism and AD in a Japanese population. We also analysed this association by stratifying subjects according to alcohol consumption and the APOE status as well as by studying patients with AD who underwent single photon emission computed tomography (SPECT). We showed the polymorphism affect the onset of young AD and specific AD patients with habitual alcohol drinking. SPECT study suggested hyperperfusion in the C allele carrier group was detected in the right inferior frontal gyrus compared with the T/T group.

Methods

Subjects

Sporadic cases of AD were diagnosed according to the NINCDS-ADRDA criteria by geriatric psychiatrists (McKhann et al. 1984). We interviewed all cases and confirmed none of them had a familial history of AD within one degree. There were no autopsy confirmed AD cases. Patients defined as the Juntendo AD (young AD) group were recruited from the Department of Psychiatry, Juntendo University Hospital, Tokyo, Japan and Department of Psychiatry Juntendo Koshigaya Hospital, Saitama, Japan. Patients defined as the Jikei AD (old AD) group were recruited from the Department of Psychiatry, Jikei University Hospital, Tokyo, Japan and the Department of Psychiatry, Jikei Kashiwa Hospital, Chiba, Japan. All the AD cases were prescribed standard medicine for the disease. Control volunteer subjects were recruited from members of the staff of our hospital with no history of dementia or other neuropsychiatric diseases.

Based on previously defined alcohol consumption standard (Mukamal et al. 2001), individuals with HAC-AD were included in the Juntendo AD (young AD) group. The HAC-AD group included 45 males and one female, and the mean age did not differ significantly from that of the control group. Our age-matched controls were for AD and did not represent as low alcohol consumption.

The purpose and significance of this study were explained to each patient in detail in writing, including verbal supplementation, as required. All subjects provided written informed consent. Table 1 shows the characteristics of the subjects. The mean age of the Juntendo AD (young AD) group did not differ significantly from that of control group. In contrast, the mean age of Jikei AD (old AD) group was significantly higher than that of the Juntendo AD (young AD) and controls groups. The gender difference between the Juntendo AD and control groups were not detected statistically (χ^2=1.44, p=0.23). The Ethics Committees of both the Juntendo

University School of Medicine and the Jikei University School of Medicine approved the study protocols.

We performed power calculations for our case-control subjects for using the Power Calculator (http://www.sph.umich.edu/csg/abecasis/CaTS/). Power was calculated under the prevalence of 0.16 using an additive or a multiplicative model, based on allelic frequencies of 0.22 with odds ratio (1.31) and an alpha level of 0.05. Results for power analyses demonstrated that the power ranged 51% from the addictive model and 56% from the multiplicative model.

Genotyping

DNA was extracted from white blood cells using a standard method. Rs17070145 was genotyped using an ABI7500 Real-Time PCR System (Applied Biosystems, CA, USA) and TaqMan technology. Probes and primers were designed by the Assay-by-Design service of Applied Biosystems. A standard PCR reaction (10 μl) was performed using the TaqMan Universal PCR Master Mix reagent kit. APOE genotypes for all samples were determined according to (Wenham et al. 1991)).

Statistical Analysis for Genetic Data

Differences in the genotypic and allelic frequencies were evaluated using a case-control study design and by applying Fisher's exact test. Hardy–Weinberg equilibrium (HWE) tests were performed in cases and controls. The case-control association and HWE analyses were performed using SNPAlyse version 7.0 Pro (Dynacom, Chiba, Japan). The combined effects of APOE and KIBRA polymorphisms on the risk for AD were tested using multinomial logistic regression analysis, and the odds ratios were calculated. The analyses were performed using the SPSS software ver. 17.0 for Windows (Chicago, Ill., USA). A p-value <0.05 was

considered statistically significant.

Brain SPECT Procedure

Twenty-three members of the Jikei AD (old AD) group laid in a supine position with their eyes closed and underwent cerebral blood flow (CBF) measurements. Each subject was intravenously administered 600 MBq of technetium-99m-ethyl cysteinate dimer (99mTc-ECD). Twenty minutes later, brain SPECT was performed using the step-and-shoot method as follows: 50 s per angle and 72 angles for 22 min. The matrix size was 128 × 128, and the location window was 140 keV at 20%. For pre-filter and absorption corrections, a ramp filter (order, 8.0; cut-off, 0.27) was used. The image voxel size was 3.2 mm. The SPECT system was a three-detector gamma camera (PRISM-IRIX; Shimadzu Medical Co., Kyoto, Japan) with a low-energy high-resolution collimator.

Statistical Analysis of Imaging Data

Data were processed using the Statistical Parametric Mapping 8 (http://www.fil.ion.ucl.ac.uk/spm) software as implemented in MATLAB 8.0 (MathWorks, Natick, MA, USA). SPECT data were normalised to the 99mTc-ECD template in the Montreal Neurological Institute (MNI) space using linear proportions and a non-linear sampling algorithm. The normalised SPECT images were then smoothed using a 12-mm full-width at half-maximum kernel.

The regional CBF (rCBF) of the T/T (16 subjects) and C allele carrier (T/C 7 subjects) groups was compared using a two-sample *t*-test. The average ages of the T/T and C allele carrier groups were not significantly different (77.2 and 77.8 years, respectively). We first set the threshold as $p < 0.005$ without correction for multiple comparisons to prevent type II errors. The extent threshold was set to 123 voxels, expected voxels per cluster. Once the

group difference was found, a post-hoc analysis was performed to investigate regional changes using small-volume correction (SVC) determined using the WFU PickAtlas software. Significance levels were set at a family-wise error (FWE) corrected $p < 0.05$. We determined the MNI coordinates to identify the anatomical region of the clusters.

Results

Genotyping

Rs17070145 was in HWE in patients with Juntendo AD (young AD), Jikei AD (old AD) and in control subjects (AD cases HWE χ^2=3.88, p=0.14 and Controls HWE χ^2=0.20, p=0.94 respectively). The frequency of the APOE4 allele in the AD groups was significantly higher compared with that in the control group (Table 1). The genotypic and allelic frequencies of rs17070145 were compared between the Juntendo AD (young AD) group and age-matched controls. Table 2 shows the genotypic frequencies of rs17070145 of each group. The genotypic and allelic frequencies of the Juntendo AD (young AD) group differed significantly from those of the control and Jikei AD (old AD) groups. Although the comparison was not statistically rigorous, the genotypic and allelic distributions of the Jikei AD (old AD) and control groups were not significantly different (data not shown).

The genotypic frequencies of rs17070145 in patients with HAC-AD were not in HWE significantly (χ^2=14.7, p=0.0005*). The number of heterozygotes was fewer than the estimated frequencies. We detected a significant association of the genotypic and allelic frequencies between HAC-AD and rs17070145 (Table 3). The frequency of the C allele in the HAC-AD group was significantly higher compared with that in the control group.

We further evaluated the relationships and interactions among the APOE genotype, rs17070145 and onset of disease, which were tested for a combinatorial effect of both polymorphisms using logistic regression tests (Table 4). We detected a significant synergistic

effect on the risk for AD from the analyses of the Juntendo AD (young AD) group. Specifically, the APOE and rs17070145 genotypes had a synergistic effect on the risk of AD [odds ratio 2.067 (95% CI, 1.410–3.030) and odds ratio 1.431 (95% CI, 1.000–2.048), respectively]. The analyses of the HAC-AD group demonstrated that the APOE genotype synergised with rs17070145 to increase the risk of HAC-AD [odds ratio 2.688, (95% CI, 1.462–4.940) and odds ratio 2.298 (95% CI, 1.361–3.878), respectively].

SPECT Analyses

Hyperperfusion in the C allele carrier group was detected in the right inferior frontal gyrus compared with the T/T group (uncorrected, $p < 0.005$). In the SVC analysis for right Brodmann areas (BAs) 46 and 47, the rCBF was significantly increased in the same brain regions at the level of FWE (corrected, $p < 0.05$). The rCBF was not significantly decreased in any region of the brain (Table 5 and Figure 1).

Discussion

We report here a genetic association between the C allele of the KIBRA rs17070145 SNP in the young [the Juntendo AD (young AD) group, mean age 65.1 ± 9.8 years] but not in the old [Jikei AD (old AD) group, mean age 77.7 ± 6.4 years] Japanese patients with AD. Regression analyses indicate that the APOE and rs17070145 genotypes acted synergistically to influence the risk of AD in the Juntendo AD (young AD) group. A study on a Japanese cohort (mean age 75.2 years) found that the rs17070145 polymorphism did not affect the risk of AD (Hayashi et al. 2010); however, the genotype and allele distribution of our Juntendo AD (old AD) group are similar to those reported by Hayashi *et al.* (2010). Discrepancies between the findings of this previous study and those of the present study (for Japanese patients) may be explained by the difference between the average ages of the study subjects.

Although the study samples for a Japanese population previously reported were not sex-matched, our case-control cohorts for young AD were sex-matched. Those differences also might affect the discrepancies.

Several similar studies that tested the association between the rs17070145 polymorphism and susceptibility to AD were conducted in other ethnic groups, including Asians (Wang et al. 2013; Hayashi et al. 2010), and compared to those, the cohort in the present study was the youngest. The results from studies on a Caucasian and an African–American cohort suggest that the T allele protects against late-onset AD (Burgess et al. 2011; Corneveaux et al. 2010). The different effects of late-onset AD in different ethnic groups might be due to the differences in allelic frequencies.

Our results show that the genotypic and allelic frequencies of rs17070145 differ significantly between the Juntendo AD (young AD) and Jikei AD (old AD) groups, suggesting an age-dependent effect. Healthy T allele carriers exhibit better episodic memory performance that is independent of age (Papassotiropoulos et al. 2006; Schaper et al. 2008). Consistent with these results are the findings of a study reporting that rs17070145 and the T allele are associated with an increased risk of the onset of AD after 86 years of age in Spanish patients with sporadic AD (Rodriguez-Rodriguez et al. 2009). They also showed age-dependent associations and C/C genotype with odds ratios that decrease continuously with age. Further, other studies (Wang et al. 2013) reported a significant interaction between the C allele and only patients with AD less who are than 74 years old, in a Han Chinese population. These results are consistent with those of the present study.

To the best of our knowledge, our present pilot study is the first to assess whether the rs17070145 polymorphism affects patients with HAC-AD. Although the genotypic and allelic distributions of controls and AD cases in a Japanese population were in HWE, those of HAC-AD were not. This suggests that the region around the polymorphism may be highly

polymorphic and could mean that the polymorphism may be triallelic; however, our results show that rs17010145 is biallelic. Alternatively, the high variability of this region may be the result of a potential large insertion or deletion in the KIBRA gene. Although, our results show that the C allele increases the risk of HAC-AD, it is possible that the data are attributable only to a small number of patients with Juntendo AD (young AD). Further, the regression analyses reveal that despite the small number of cases, the odds ratio of the association of the C allele with patients with HAC-AD cases was higher than that of the allele with patients with Juntendo AD (young AD). The HWE of patients with HAC-AD was different from that of patients with AD. Thus, we consider that the C allele is an independent risk factor of HAC-AD. Moreover, although positive associations were observed, the size of our HAC-AD cohort was relatively small, and the subjects were predominantly men. Although the logistic regression analyses showed synergistic effects of the KIBRA polymorphism and APOE4, odds ratio of the KIBRA polymorphism was lower than that of APOE4. Further genetic studies with a larger case-control cohort and the inclusion of more women are warranted.

There is evidence of an association between the KIBRA polymorphism and early relapse with substance abuse (Bauer et al. 2012). The T allele was associated with increased relapse in patients consuming alcohol and using cocaine, heroin, opiates and benzodiazepines. Another study indicates that the T allele is associated with diminished cognitive flexibility (Zhang et al. 2009). The average age of subjects of these two studies was approximately 40 years. A study on young and middle-aged volunteers indicated that T allele carriers exhibit better memory performance compared with non-carriers (Papassotiropoulos et al. 2006). Therefore, the genetic effects of KIBRA on the cognitive performance of a middle-aged healthy population are controversial. We suggest therefore that cognitive impairment in middle age causes alcohol dependencies and increases the risk of HAC-AD. Further longitudinal and prospective studies that attempt to correlate these characteristics with the

KIBRA polymorphism may clarify these relationships.

Our neuroimaging study reveals hyperperfusion in the right inferior frontal gyrus, BA 46 and BA 47, in patients carrying the KIBRA C allele. These regions are associated with working memory, executive functions and emotional inhibition. A meta-analysis showed that the KIBRA polymorphism is associated with working memory (Milnik et al. 2012). Atrophy of the inferior frontal gyrus is more prominent in patients with early-onset AD compared with those with late-onset AD (Moller et al. 2013) A SPECT study found that alcohol dependencies correlate with the inferior frontal gyrus (Noel et al. 2001). Hyperperfusion in the right inferior frontal gyrus may reflect compensatory reactions caused by dysfunctions of dopaminergic neurotransmission associated with the pathophysiologies of AD and alcoholism. Moreover, observed hyperperfusion in the right inferior frontal gyrus might associate with several behavioral and psychological symptoms of dementia (BPSD), such as aggression and agitation. Although we were unable to perform SPECT analysis of patients with Juntendo AD (younger AD) or HAC-AD in the present study, we suggest that the KIBRA C allele contributes to dysfunctions of the inferior frontal gyrus in younger patients with AD and HAC-AD. Since we could not include C/C cases for the SPECT study, gene dose effect would be studied in the future.

Several limitations should be discussed about our cohorts. Since our case-control cases were composed of two facilities that different clinical groups of geriatric psychiatrists evaluated each case, we did not combine cases. Although we think that genetic backgrounds of old AD differ from that of young AD, age-matched controls for old AD were not obtained in the study. Both AD and HAC-AD were diagnosed at one time, longitudinal observation would be needed to ascertain other dementias. As AD is heterogeneous, we focused on specific phenotypes associated with habitual alcohol use and the KIBRA genotype. Although our HAC-AD cases did not show typical alcohol dependency, such as; do not stop drinking,

increase amounts of alcohol and show behavior related with alcohol abuse, it is difficult to distinguish typical alcohol dependency from habitual drinking. Genetic association between typical alcoholic dementia and the polymorphism should be needed.

Our findings suggested the C allele of rs17010145 increase the risk for young AD and HAC-AD. The SPECT study on AD showed that the polymorphism associates with hyperperfusion in the right inferior frontal gyrus and with dysfunctions of dopaminergic neurotransmission.

Acknowledgements

This study was partially supported by the High Technology Research Center Grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by the Sportology Center, Juntendo University Graduate School of Medicine. We are grateful for the technical assistance of K. Yamamoto.

The purpose and significance of this study were explained in detail in writing, including verbal supplementation as required, to each patient and their spouse or first-degree relatives. All subjects provided their written informed consent. If the ability of the patients to provide consent were compromised, their spouse or first-degree relative provided consent. Patients without accompanying spouses or relatives were not included in this study. The Ethics Committees of the Juntendo University School of Medicine and the Jikei University School of Medicine approved the study protocols.

Conflict of Interest

We have no potential conflicts.

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 in later life, but does not increase the risk of mild cognitive impairment. *Journal of cellular and molecular medicine, 12*(5A), 1672-1676.

Bates, T. C., Price, J. F., Harris, S. E., Marioni, R. E., Fowkes, F. G., Stewart, M. C., et al. (2009). Association of KIBRA and memory. *Neuroscience letters, 458*(3), 140-143.

Bauer, L. O., Covault, J., & Gelernter, J. (2012). GABRA2 and KIBRA genotypes predict early relapse to substance use. *Drug and alcohol dependence, 123*(1-3), 154-159.

Brouwers, N., Sleegers, K., & Van Broeckhoven, C. (2008). Molecular genetics of Alzheimer's disease: an update. *Annals of medicine, 40*(8), 562-583.

Burgess, J. D., Pedraza, O., Graff-Radford, N. R., Hirpa, M., Zou, F., Miles, R., et al. (2011). Association of common KIBRA variants with episodic memory and AD risk. *Neurobiology of aging, 32*(3), 557 e551-559.

Corneveaux, J. J., Liang, W. S., Reiman, E. M., Webster, J. A., Myers, A. J., Zismann, V. L., et al. (2010). Evidence for an association between KIBRA and late-onset Alzheimer's disease. *Neurobiology of aging, 31*(6), 901-909.

Duning, K., Wennmann, D. O., Bokemeyer, A., Reissner, C., Wersching, H., Thomas, C., et al. (2013). Common exonic missense variants in the C2 domain of the human KIBRA protein modify lipid binding and cognitive performance. *Translational psychiatry, 3*, e272.

Frisardi, V., Solfrizzi, V., Seripa, D., Capurso, C., Santamato, A., Sancarlo, D., et al. (2010). Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease. *Ageing research reviews, 9*(4), 399-417.

Hayashi, N., Kazui, H., Kamino, K., Tokunaga, H., Takaya, M., Yokokoji, M., et al. (2010). KIBRA genetic polymorphism influences episodic memory in Alzheimer's disease, but does not show association with disease in a Japanese cohort. *Dementia and geriatric cognitive disorders, 30*(4), 302-308.

Kauppi, K., Nilsson, L. G., Adolfsson, R., Eriksson, E., & Nyberg, L. (2011). KIBRA polymorphism is related to enhanced memory and elevated hippocampal processing. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 31*(40), 14218-14222.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology, 34*(7), 939-944.

Milnik, A., Heck, A., Vogler, C., Heinze, H. J., de Quervain, D. J., & Papassotiropoulos, A. (2012). Association of KIBRA with episodic and working memory: a meta-analysis. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics, 159B*(8), 958-969.

Moller, C., Vrenken, H., Jiskoot, L., Versteeg, A., Barkhof, F., Scheltens, P., et al. (2013). Different patterns of gray matter atrophy in early- and late-onset Alzheimer's disease. *Neurobiology of aging, 34*(8), 2014-2022.

Mukamal, K. J., Longstreth, W. T., Jr., Mittleman, M. A., Crum, R. M., & Siscovick, D. S. (2001). Alcohol consumption and subclinical findings on magnetic resonance imaging of the brain in older adults: the cardiovascular health study. *Stroke; a journal of cerebral circulation, 32*(9), 1939-1946.

Need, A. C., Attix, D. K., McEvoy, J. M., Cirulli, E. T., Linney, K. N., Wagoner, A. P., et al. (2008). Failure to replicate effect of Kibra on human memory in two large cohorts of

European origin. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics, 147B*(5), 667-668.

Noel, X., Paternot, J., Van der Linden, M., Sferrazza, R., Verhas, M., Hanak, C., et al. (2001). Correlation between inhibition, working memory and delimited frontal area blood flow measure by 99mTc-Bicisate SPECT in alcohol-dependent patients. *Alcohol Alcohol, 36*(6), 556-563.

Ohta, S., & Ohsawa, I. (2006). Dysfunction of mitochondria and oxidative stress in the pathogenesis of Alzheimer's disease: on defects in the cytochrome c oxidase complex and aldehyde detoxification. *Journal of Alzheimer's disease : JAD, 9*(2), 155-166.

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

Preuschhof, C., Heekeren, H. R., Li, S. C., Sander, T., Lindenberger, U., & Backman, L. (2010). KIBRA and CLSTN2 polymorphisms exert interactive effects on human episodic memory. *Neuropsychologia, 48*(2), 402-408.

Reitz, C., & Mayeux, R. (2014). Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochemical pharmacology, 88*(4), 640-651.

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*(2), 322-324.

Schaper, K., Kolsch, H., Popp, J., Wagner, M., & Jessen, F. (2008). KIBRA gene variants are associated with episodic memory in healthy elderly. *Neurobiology of aging, 29*(7), 1123-1125.

Schneider, A., Huentelman, M. J., Kremerskothen, J., Duning, K., Spoelgen, R., & Nikolich, K. (2010). KIBRA: A New Gateway to Learning and Memory? *Frontiers in aging neuroscience, 2*, 4.

Sedille-Mostafaie, N., Sebesta, C., Huber, K. R., Zehetmayer, S., Jungwirth, S., Tragl, K. H., et al. (2012). The role of memory-related gene polymorphisms, KIBRA and CLSTN2, on replicate memory assessment in the elderly. *J Neural Transm, 119*(1), 77-80.

Vassos, E., Bramon, E., Picchioni, M., Walshe, M., Filbey, F. M., Kravariti, E., et al. (2010). Evidence of association of KIBRA genotype with episodic memory in families of psychotic patients and controls. *Journal of psychiatric research, 44*(12), 795-798.

Vyas, N. S., Ahn, K., Stahl, D. R., Caviston, P., Simic, M., Netherwood, S., et al. (2014). Association of KIBRA rs17070145 polymorphism with episodic memory in the early stages of a human neurodevelopmental disorder. *Psychiatry research, 220*(1-2), 37-43.

Wang, H. F., Tan, L., Yu, J. T., Ma, X. Y., Liu, Q. Y., & Wang, W. (2013). Age-dependent association of KIBRA gene polymorphism with Alzheimer's disease in Han Chinese. *Molecular biology reports, 40*(12), 7077-7082.

Wenham, P. R., Price, W. H., & Blandell, G. (1991). Apolipoprotein E genotyping by one-stage PCR. *Lancet, 337*(8750), 1158-1159.

Wilker, S., Kolassa, S., Vogler, C., Lingenfelder, B., Elbert, T., Papassotiropoulos, A., et al. (2013). The role of memory-related gene WWC1 (KIBRA) in lifetime posttraumatic stress disorder: evidence from two independent samples from African conflict regions. *Biological psychiatry, 74*(9), 664-671.

Yasuda, Y., Hashimoto, R., Ohi, K., Fukumoto, M., Takamura, H., Iike, N., et al. (2010). Association study of KIBRA gene with memory performance in a Japanese population. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry, 11*(7), 852-857.

Zhang, H., Kranzler, H. R., Poling, J., Gruen, J. R., & Gelernter, J. (2009). Cognitive

flexibility is associated with KIBRA variant and modulated by recent tobacco use. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 34*(12), 2508-2516.

Figure 1

Regions with significant hyperperfusion in patients with the rs17070145 T/C genotype compared with those with the T/T genotype ($p < 0.05$ with small volume correction). Significantly increased hyperperfusion in the C allele carrier group compared with the T/T group was detected in the right inferior frontal gyrus. No significant hypoperfusion was observed.

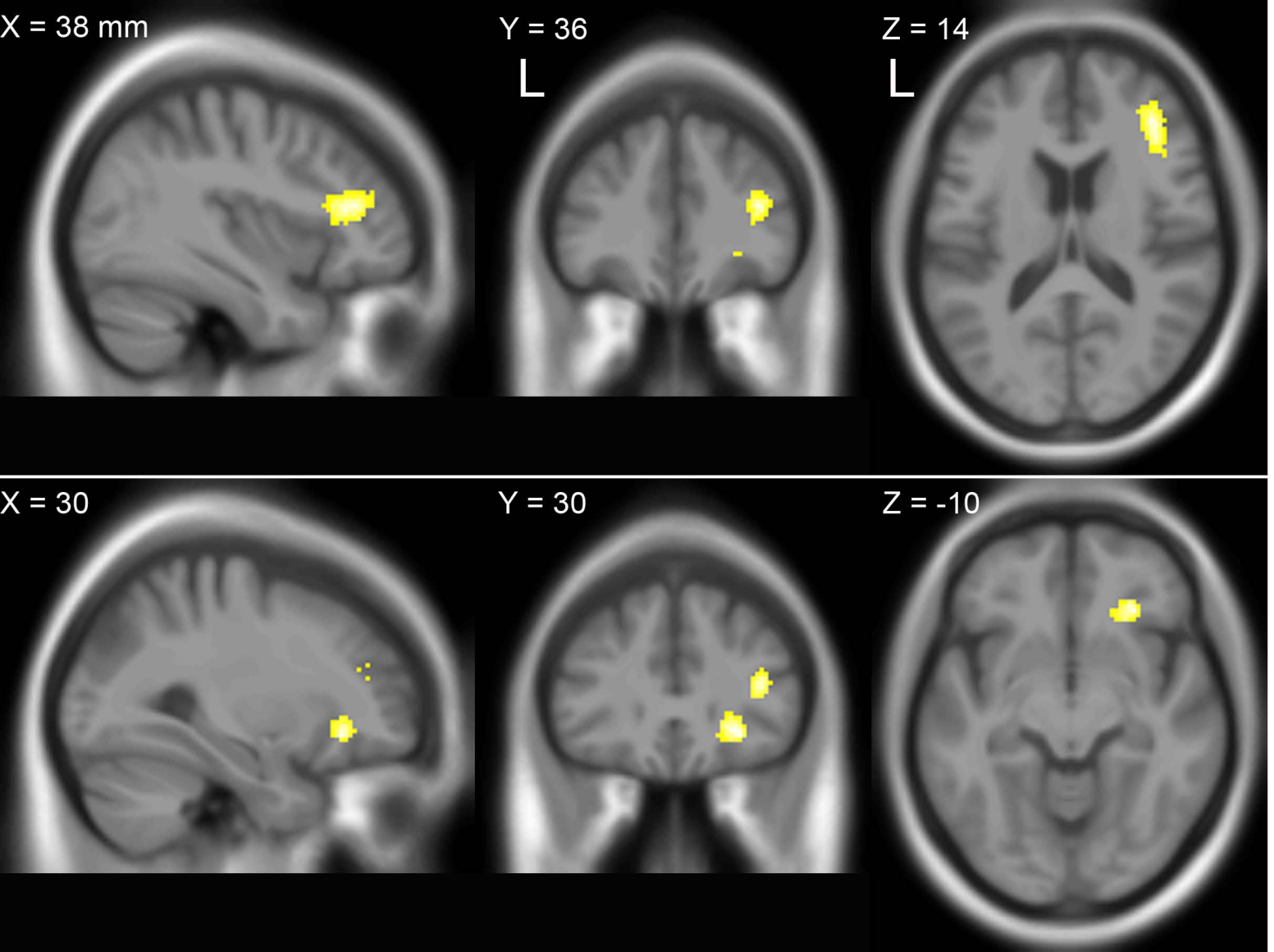
X = 38 mm
Y = 36
L
Z = 14
L
X = 30
Y = 30
Z = -10
T value
4
3.5
3
2.5
2
1.5
1
0.5
0

Table 1. Characteristics of subjects

Group	N (male:female)	Mean age ± SD	APOE4-positive cases
Control	154 (71:83)	64.0 ± 6.8	41 (26.6%)
Juntendo AD	237 (124:113)	65.1 ± 9.8	99 (41.8%)
HAC-AD	46 (45:1)	65.2 ± 10.0	20 (43.5%)
Jikei AD	160 (50:110)	77.7 ± 6.4	76 (47.5%)

HAC-AD, high alcohol consumption-Alzheimer's disease; SD. standard deviation; APOE4, apolipoprotein E 4

Juntendo AD: young AD

Jikei AD: old AD

Table 2. Frequencies of the KIBRA rs17070145 genotype and its alleles

KIBRA rs17070145	Genotype T/T	T/C	C/C		Odds ratio (95% CI)
Control	108 (0.70)	43 (0.28)	3 (0.02)		
Juntendo AD	157 (0.66)	58 (0.24)	22 (0.09)	Control vs Juntendo AD	
				$\chi^2 = 8.49$, $p = 0.01$*	
Jikei AD	105 (0.66)	51 (0.32)	4 (0.02)	Juntendo AD vs. Jikei AD	
				$\chi^2 = 8.62$, $p = 0.01$*	
	T allele	C allele			
Control	259 (0.84)	49 (0.16)			
Juntendo AD	372 (0.78)	102 (0.22)		Control vs Juntendo AD	0.690
				$\chi^2 = 3.77$, $P = 0.05$*	(0.460–1.005)
Jikei AD	261 (0.82)	59 (0.18)		Juntendo AD vs Jikei AD	0.824
				$\chi^2 = 1.12$, $P = 0.29$	(0.572–1.165)

HWE, Hardy–Weinberg equilibrium; AD, Alzheimer's disease; CI, confidence interval.

*Statistically significant (Fisher's exact test).

Juntendo AD: young AD

Jikei AD: old AD

Table 3. Analysis of patients with HAC-AD for the KIBRA rs17070145 genotype and allele frequencies

KIBRA	Genotype			χ^2	*P*	Odds ratio (95% CI)
rs17070145	T/T	T/C	C/C			
Control	108 (0.70)	43 (0.28)	3 (0.02)			
HAC-AD	30 (0.65)	4 (0.09)	12 (0.26)	33.21	0.00005*	
	T allele	C allele				
Control	259 (0.84)	49 (0.16)				
HAC-AD	64 (0.70)	28 (0.30)		9.62	0.0004*	2.312 (1.274–3.858)

HAC-AD, High alcohol consumption-Alzheimer's disease; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

*Statistically significant (Fisher's exact test).

Table 4. Logistic regression analysis to detect an association among the APOE genotype, KIBRA rs17070145 and the onset of AD

(Control vs. Juntendo AD)	B	*P*	Odds ratio (95% CI)
APOE4	0.726	0.0001*	2.067 (1.410–3.030)
KIBRA C allele	0.359	0.05*	1.431 (1.000–2.048)
			*model χ^2 test <0.01
			Discrimination rate: 60.6%
(Control vs. HAC-AD)	B	*P*	Odds ratio (95% CI)
APOE4	0.989	0.001*	2.688 (1.462–4.940)
KIBRA C allele	0.832	0.002*	2.298 (1.361–3.878)
			*model χ^2 test <0.01
			Discrimination rate: 79.0%

APOE4, apolipoprotein E 4; CI, confidence interval; HAC-AD, High alcohol consumption-Alzheimer's disease

Table 5. SPM results showing MNI coordinates and significance levels of brain regions with hyperperfusion in the T/C group than the T/T group

Region	Coordinates (mm)					SVC
	x	y	z	peak t	cluster size	FWE-corrected *p*-value
Right inferior frontal gyrus						
BA 46	38	36	14	4.09	281	0.042
BA 47	30	30	−10	4.24	145	0.04

BA, Brodmann Area