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Association of a *CAMK2A* genetic variant with logical memory performance and hippocampal volume in the elderly

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Short title: CAMK2A and logical memory in the elderly

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Highlights

- Calcium/Calmodulin-dependent kinase alpha is essential for learning and memory
- we tested the association of CAMK2A SNPs with verbal logical memory in elderly people with cognitive impairments
- rs919741 predicted a higher hippocampal volume and better logical memory in the elderly
- no such association was found in healthy adults

Abstract

Calcium/Calmodulin-dependent kinase alpha (αCaMKII) has been shown to play an essential role in synaptic plasticity and in learning and memory in animal models. However, there is little evidence for an involvement in specific memories in humans. Here we tested the potential involvement of the αCaMKII coding gene *CAMK2A* in verbal logical memory in two Caucasian populations from Germany, in a sample of 209 elderly people with cognitive impairments and a sample of 142 healthy adults. The association of single nucleotide polymorphisms (SNPs) located within the genomic region of *CAMK2A* with verbal logical memory learning and retrieval from the Wechsler Memory Scale was measured and hippocampal volume was assessed by structural MRI. In the elderly people, we found the minor allele of *CAMK2A* intronic SNP rs919741 to predict a higher hippocampal volume and better logical memory retrieval. This association was not found in healthy adults. The present study may provide evidence for an association of a genetic variant of the *CAMK2A* gene specifically with retrieval of logical memory in elderly humans. This effect is possibly mediated by a higher hippocampal volume.

Keywords: αCaMKII, CAMK2A, logical memory, hippocampus, elderly, adults

Introduction

Learning and memory performance are crucial determinants of everyday activities. During aging, a decline in learning and memory capacity is observed in humans that may end up in dementia (Erickson and Barnes, 2003). Inter-individual differences based on genetic predispositions are now emerging that may allow a prognosis of learning and memory performance in the elderly, for which molecular mechanisms in the brain have been described (De Souza Silva et al., 2013). While neuroscience has identified numerous networks underlying learning and memory, less is known about how natural variations contribute to human performance.

Among the Calcium/Calmodulin (Ca^{2+}/CaM)-dependent kinases (CaMKs), CaMKII have been shown to play an important role for the formation of long term potentiation (LTP) and –depression (LTD) and learning (Lisman et al., 2012; Giese and Mizuno, 2013; Bayer and Schulman, 2019). It is a very abundant group of proteins in the brain (Hanson and Schulman, 1992). CaMKIIs are holoenzymes that consist of 12-14 subunits (Rosenberg et al., 2005). αCaMKII and βCaMKII are the most abundant CaMKII subunits in the brain. αCaMKII is predominantly expressed in glutamatergic neurons (Liu and Jones, 1996), while βCaMKII may be expressed in inhibitory and excitatory neurons (Borgesius et al., 2011; Lamsa et al., 2007). αCaMKII activity can be regulated by the autophosphorylation at the threonine-286 (T286) site, which results from an inter-subunit kinase reaction within the holoenzyme. T286-autophosphorylation switches the kinase from Ca^{2+}/CaM-dependence to independence (Irvine et al., 2006). The autophosphorylation of αCaMKII prolongs its kinase activity at the synapse even beyond the Ca^{2+} transient. This has been suggested as a ‘molecular memory’ for this transient that may be one of the key mechanisms for rapid learning, but is less important for

memory (Irvine et al., 2005). An important role of αCaMKII has been also demonstrated for maladaptive memory formation leading to psychiatric disorder symptoms in animals (Fog et al., 2006; Easton et al., 2013a, 2013b; 2014; Steinkellner et al., 2014; Schöpf et al., 2015). Previously, we have shown an association of a single nucleotide polymorphism (SNP) in the αCaMKII coding gene *CAMK2A* which predicts working memory performance in a population of adolescents and adults (Easton et al. 2013c). A more recent study showed that various *de novo* mutations affecting the *CAMK2A* gene may result in reduced αCaMKII activity leading to intellectual disability, including severe learning and memory deficits (Küry et al., 2017; Chia et al., 2018). However, also de novo mutations leading to enhanced αCaMKII autophosphorylation may result in epilepsy and neurodevelopmental retardation (Akita et al., 2018).

One of the most important associative memory domains in humans is the logical memory (Wang and Cui, 2018), where information has to be learned in logical order and retrieved as composite information. This memory capacity declines particularly with aging having a severe impact on independency and quality of life in the elderly. In the present study we analyzed whether genetic variants of *CAMK2A* may predict logical memory performance in elderly and adult humans. We hypothesized associations between *CAMK2A* SNPs and Wechsler memory scale (WMS) performance in immediate and/or delayed recall of verbal logical memories, as well as with hippocampal volumes.

1. Materials and Methods

1.1. Experiment I: Association analysis in elderly patients

A sample of 209 elderly patients (n=91 males, n=118 females) with cognitive impairments (n=59 mild cognitive impairment, n=150 dementia) was investigated. The participants were recruited within the early diagnostic and prognostic program of the Competence Network on Dementia (CND) at each of the 13 participating sites in Germany with the diagnosis of mild

cognitive impairment or Alzheimer's disease (Kornhuber et al., 2009; Jessen et al., 2009). Participants were between 50-90 years old (71.4±0.6; mean±SEM). All patients were tested for logical memory subtest of the Wechsler Memory Scale (WMS) for immediate and delayed retrieval of a verbal story (Abikoff et al., 1987).

All patients were genotyped using the Illumina Omni-1M-Quad array. Forty-two SNPs were situated around the *CAMK2A* gene (University of California, Santa Cruz genome browser https://genome.ucsc.edu; GRCh37/hg19; NM_015981, NM_171825; chr5:149599054-149669403 [predefined region of interest: chr5:149589054-149674403]). Eight SNPs were excluded due to a minor allele frequency (MAF) <0.05 (rs6873782, rs2286643, rs11950723, rs6869180, rs882663, rs10053906, rs12109550) or a significant deviation from Hardy-Weinberg equilibrium (HWE) (rs3806948) leaving 34 SNPs (Fig. 1). In addition, five SNPs were excluded because of linkage disequilibrium (LD) of $R^2 \geq 0.8$ (rs17111051, rs7732895, kgp9208102, rs17656349, rs6885505). MAFs, HWE and LD structure were analyzed using Haploview version 4.2 (https://www.broadinstitute.org/haploview/haploview/; LD plot; Fig. 1). In the end, we tested 29 SNPs for their associations with logical memory performance and hippocampal volumes.

1.2. Experiment II: Association analysis in adults

A sample of 140 young healthy adults (n=48 males, n=92 females) was analyzed within the GENES study at the Friedrich-Alexander University Erlangen-Nürnberg (Rhein et al., 2014, Richter-Schmidinger et al., 2011; Alexopoulos et al., 2011; Mühle et al., 2016). Participants were between 19 and 35 years old (mean age 25 years). All participants were tested for delayed reproduction of a verbal story (Richter-Schmidinger et al., 2011) using the IGD (*Inventar zur Gedächtnisdiagnostik*, IGD) (Baller et al. 2006).

Genomic DNA was isolated from whole blood using the Gentra Puregene Blood Kit (Qiagen) according to the supplier's protocol. Genotyping of the *CAMK2A* rs919741 polymorphism that was identified in the elderly sample was performed using high resolution melting (HRM, (Wittwer et al. 2003) on a Roche LightCycler 480. For the HRM reaction, a polymerase chain reaction was set up containing 10 ng genomic DNA in 10 mM Tris/HCl pH 8.9, 50 mM KCl, 0.02% Tween-20, 2.5 mM $MgCl_2$, 200 µM dNTPs, 200 nM forward (GCTTGGATTCTGCTCACTATGT) and reverse (TGTAAAATGAGGACACCACCA) primers and 0.1 unit *Taq* DNA polymerase (Rovalab) in a total volume of 10 µl. Moreover, 0.5 µl EvaGreen (Biotium, 20x) were added to the reaction to allow real-time quantification and subsequent detection of the melting curve by fluorescence analysis. After initial denaturation at 95°C for 2 min, the template was amplified during 40 cycles of 12 s denaturation at 96°C, 15 s annealing at 58°C and 15 s extension at 72°C followed by a denaturation (2 min 95°C) and a re-annealing step (40°C). The 67 bp products were then slowly melted from 70-85°C under high resolution fluorescence recording. Quantitative evaluation and genotype analyses were performed with gene scanning software (Roche).

Data of samples not fulfilling a set of quality criteria with respect to Cq values or fluorescence levels were neglected, and the analysis was repeated. The correct assignment of melting curves to genotypes was confirmed by restriction fragment length analysis using *Bcc*I to cleave HRM reaction products originating from the minor A allele. Furthermore, 28% of the sample set was repeated to confirm the genotype with a reproducibility of 100%. The obtained genotype frequencies (heterozygosity rate of 0.486) were in agreement with the Hardy-Weinberg equilibrium ($p>0.05$) and very close to the known frequencies for Utah residents with Northern and Western European ancestry from the CEPH collection population (CEU) from the HapMap Genome Browser (0.434).

1.3. Magnet resonance imaging

Experiment I: Hippocampus volume was measured by structural volumetric magnet resonance imaging (MRI) in 111 of the elderly participants, as described previously (Ewers et al., 2006; Teipel et al., 2006; De Souza Silva et al. 2013).

Experiment II: To measure the hippocampal volumes in 140 healthy young adults, three-dimensional structural MRI scans were acquired on a 1.5-Tesla scanner (Siemens Sonata, Erlangen, Germany), processed and evaluated using the FreeSurfer 5.1.0 software as previously described (Rhein et al., 2014; Mühle et al., 2017).

1.4. Statistical analysis

Experiment I: Because the WMS values deviated significantly from normal distribution according to the Kolmogorov-Smirnov test ($p<0.005$), they were transformed into ranks (Conover and Iman, 1981), resulting in values for skewness and excess kurtosis between -1.5 and 1.5. Linear regression models (ENTER method) were used to calculate the associations between each of the 29 SNPs (general genetic model [homozygous major allele=1, heterozygous=2, homozygous minor allele=3]; allelic model [major allele vs. minor allele]) and WMS (immediate/delayed) performance. We then investigated whether the initially discovered SNP rs919741 was also related to the hippocampal volume. Hierarchical linear regression analyses were used to estimate the contribution of hippocampal volume to the relationship between the identified SNP rs919741 and neuropsychological test performance. In model 1, the genetic data were entered first and hippocampal volumes were entered second. In model 2, hippocampal volumes were entered first and the genetic data second. We adjusted all models for age, sex and mild cognitive impairment *vs.* dementia status. A $p_a<0.05$ was considered nominally significant. Because of the numerous tests and the small cohort size, we calculated 95%- confidence intervals (CIs) and p-values (p_b) using bootstrap (10,000 resamples) to validate our findings. P-values were corrected according to Bonferroni (p_c).

Subsequently, we also compared rs919741 AA/GA subjects with GG subjects in terms of ranked WMS delayed recall scores and hippocampal volumes using Student's t-tests.

Experiment II: IGD values deviated significantly from normal distribution according to the Kolmogorov-Smirnov test ($p<0.05$). To assess differences in memory values between the three different genotypes, we applied the non-parametric Kruskal-Wallis test. Differences in hippocampal volume were assessed using ANOVA. A $p<0.05$ was considered statistically significant. IBM SPSS Statistic 20.0 and Graph Pad Prism 4 (Graph Pad Software Inc., USA) were used for statistical analyses and illustrations of both experiments.

2. Results

2.1. Association of the CAMK2A variant rs919741 with logical memory in the elderly

The statistical analyses revealed nominally significant associations of immediate and delayed recall WMS logical memory performance with rs980272, rs6869490, rs957709, rs2241694, rs4958469, rs919741, and rs6869634 (Tab. 1). After correction for multiple testing (Bonferroni), however, there was no effect of the investigated *CAMK2A* SNPs on immediate recall WMS performance (Tab. 1; Fig. 2A), but rs919741 remained significantly related to WMS delayed recall (Fig. 2B; general genetic model: $B=21.67$, $p_a=7.0x10^{-04}$, 95%-CI 8.90-33.59, $p_b=4.0x10^{-04}$, $p_c<0.05$; allelic model: $B=21.44$, $p_a=7.6x10^{-04}$, 95%-CI 8.73-33.91, $p_b=1.4x10^{-03}$). Individuals homozygous for the minor allele (AA, n=10, 4.8%) performed better than heterozygous individuals (GA, n=73, 34.9%) who performed better than individuals homozygous for the major allele (GG, n=126, 60.3%). The analysis of effect size yielded a Cohens d=0.27 with a significantly higher score in AA/GA subjects vs. GG/GA subjects (Student's t-test on ranked WMS delayed recall, t=-2.6, p=0.011). The two models were also significantly affected by age (higher WMS delayed recall score related to younger

age; general genetic model: B=-1.18, p_a=8.7x10^{-03}, 95%-CI -2.04--0.03, p_b=9.0x10^{-03}; allelic model: B=-2.36, p_a=2.3x10^{-04}, 95%-CI -3.58--1.10, p_b=1.0x10^{-04}) and mild cognitive impairment vs. dementia status (higher delayed WMS recall score in mild cognitive impairment; general genetic model: B=-27.96, p_a=8.6x10^{-04}, 95%-CI -45.08--11.30, p_b=1.5x10^{-03}; allelic model: B=-54.79, p_a=4.0x10^{-06}, 95%-CI -79.46--30.72, p_b=2.0x10^{-04}) (Fig. 3).

2.2. Association of the CAMK2A variant rs919741with hippocampal volume in the elderly

Subsequently, we found that rs919741 was significantly related to total, right and left hippocampal volumes (Fig. 2C; general genetic model: total, B=450.41, p_a=6.4x10^{-04}, 95%-CI 232.67-665.65, p_b=4.0x10^{-04}, right, B=207.33, p_a=7.6x10^{-03}, 95%-CI 75.34-347.33, p_b=3.4x10^{-03}, left B=243.09, p_a=8.0x10^{-04}, 95%-CI 103.81-379.71, p_b=1.2x10^{-03}; allelic model: total, B=217.33, p_a=8.1x10^{-04}, 95%-CI 102.59-335.02, p_b=4.0x10^{-04}, right, B=100.04, p_a=8.5x10^{-03}, 95%-CI 32.98-168.64, p_b=5.1x10^{-03}, left B=117.30, p_a=1.0x10^{-03}, 95%-CI 48.01-186.91, p_b=1.0x10^{-03}). The analysis of effect size yielded Cohen's d effect sizes of 0.62, 0.55, and 0.56 with significantly higher volumes in AA/GA subjects vs. GG subjects (Student's t-tests on total hippocampal volume: t=-3.2, p=0.002, right hippocampus: t=-2.8, p=0.005, left hippocampus: t=-2.9, p=0.004).

The hierarchical linear regression analyses (general genetic model) showed that rs919741 explained 9.2% of the WMS performance variance, 2.3% of which was explained by total hippocampal volume. This suggests that about 25% (2.3%/9.2%) of the association between rs919741 and delayed WMS performance can be explained by total hippocampal volume.

2.3. CAMK2A association with logical memory and hippocampal volume in adults

Due to the strong association of the *CAMK2A* SNP rs919741 with delayed recall memory in the elderly, we investigated if this effect holds also true in young healthy adults. In this group, the rs919741 genotype was not associated with better logical memory (p=0.502, Fig. 4A) and hippocampal volume (p=0.595) (Fig. 4B). It should be noted that in our adult sample a very uneven haplotype distribution emerged, with the elderly protective rs919741 AA genotype only present at a 4/140 (3%) rate (GA, n=60, 43 %; GG n=76, 54%). This might limit the statistical analysis.

3. Discussion

αCaMKII and its signaling is one of the best known molecular pathway involved in LTP/LTD and the formation of associative memories (Giese and Mizuno, 2013; Lisman, 2017; Bayer and Schulman, 2019). A complete lack of function or autophosphorylation enhancing gain of function induced by *de novo* mutations of the *CAMK2A* gene at various sites may lead to neurodevelopmental impairments and severe intellectual disabilities (Küry et al., 2017; Akita et al., 2018). However, little is known about how αCaMKII and its regulation are involved in normal human associative learning and memories. Here we report a significant association of the *CAMK2A* rs919741 SNP with logical memory in a population of elderly people with cognitive impairments from Germany. In this population, the rs919741 AA genotype showed a better delayed recall in a verbal logical memory test. This genotype was also associated with higher hippocampal volume which may explain more efficient memory performance. In a second sample, we investigated whether this genotype may also be predictive for better logical memory performance and hippocampal volume in a sample of young healthy adults. The results did not point to an association between genotype, logical memory and hippocampal volume in young healthy people. Thus, this association might emerge with increasing age.

In a previous study in two healthy Caucasian adult populations unrelated to the present study we found a significant association with of *CAMK2A* SNPs with spatial and non-spatial working memory. In this sample of adolescents, of the 31 *CAMK2A* SNPs genotyped, three were found to be significantly associated with task performance in the spatial working memory strategy score of the CANTAB test battery: rs874083, rs7701427, and rs10463293. A structural MRI analysis did not show any association between *CAMK2A* SNPs and volume, thickness, or surface area in 14 frontal cortex subregions or the hippocampus in this sample. This might suggest that working memory performance differences were not mediated by altered capacity of task relevant brain circuits (Easton et al., 2013c). In order to confirm this association, a sample of Caucasian adults from Switzerland was investigated and showed a significant association of *CAMK2A* SNP rs34087853 with non-spatial working memory performance. SNP rs34087853 was in significant, albeit rather weak, LD with the first discovered SNP rs874083, which is located 6 kb distant to rs34087853 (Easton et al., 2013c). Although these SNPs were mostly covered in the present study, no overlap appeared in the associations of *CAMK2A* variants with spatial/non-spatial working memory or with verbal logical memory. In the present study these associations emerged mainly in a population of elderly people with cognitive impairments. Only the low population penetrance of the protective AA genotype may currently prevent a generalization of the protective genotype to a wider age span.

While a previous study did not suggest that the association of *CaMK2A* polymorphisms and memory could be mediated by altered structural properties of the brain (Easton et al., 2013c), the current study might implicate that. Here, the rs919741 AA genotype did not only predict a better delayed recall of logical memories, but also a higher total hippocampal volume. Since the hippocampus is a crucial structure for associative learning processes (Dere et al., 2007; Wang and Cui, 2018), this structural association might mediate the functional association (Smolen et al., 2019).

CaMKII comprises 28 similar isoforms which are derived from four genes, α, β, γ and δ. In the brain, α- and β-subunits are the predominant CaMKII isoforms with the α-isoform being the most abundant in the forebrain, forming αCaMKII holoenzymes. Thereby, αCaMKII oligomerization through the association domains is required for substrate phosphorylation, synaptic localization and learning and memory (Giese and Mizuno, 2013; Bhattacharyya et al., 2016). Each αCaMKII contains a catalytic domain, an autoinhibitory domain, a variable segment, and a self-association domain. Importantly, there is a region within the autoinhibitory domain that resembles the protein substrate of the catalytic domain. Under basal conditions, when the intracellular concentration of the second messenger Ca^{2+} is maintained at a very low level, αCaMKII appears to be almost inactive. Significant activation, which involves alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) receptor stimulation, raises intracellular Ca^{2+} levels. A Ca^{2+} transient and subsequent Ca^{2+}/CaM activation, which follow NMDA-receptor stimulation, can activate αCaMKII. But it will become inactive again in less than 1 sec. In the presence of Ca^{2+} activation, however, the Ca^{2+}/CaM complex binds to αCaMKII and induces a conformational change which exposes the catalytic domain. At the same time Thr286 on the autoinhibitory domain is exposed, which then can be phosphorylated by the neighboring subunit. When Ca^{2+} levels fall and Ca^{2+}/CaM dissociates from the kinase, it would normally be rendered inactive. However, as long as the Thr286 site remains phosphorylated αCaMKII remains active even in the absence of Ca^{2+}. Accordingly, the αCaMKII autophosphorylation prevents the enzyme from switching back into an inactive stage. The resulting Ca^{2+} independent activity is considered to be a molecular memory of the previous Ca^{2+} activation that serves as a critical mechanism for memory at the behavioural level (Giese and Mizuno, 2013; Bayer and Schulman, 2019). The complex nature of the molecule and its many

functional sites suggest that various *de novo* mutations also in non-coding introns, as reported here, may affect parts of its function.

Küry and colleges (2017) reported 19 different heterozygous variants in *CAMK2A* or *CAMK2B* in 24 unrelated individuals with intellectual disabilities. The *de novo* status of the mutation was confirmed for 18 out of 19 variants. For most of these alterations, protein and neuronal dysfunctions were identified. Eight of those missense mutations affected the catalytic domain of the enzyme, while all others affected the regulatory domain. Thereby, intellectual disabilities were more profound when the autoregulatory domain of αCaMKII was affected compared to the kinase domain. Interestingly, these mutations did not only induce intellectual disabilities, but also a number of other developmental disorders, such as delayed speech and language development, delayed gross motor development, visual impairments, and abnormal emotion and affective behavior (Küry et al., 2017). The later was previously suggested in mice studies (Easton et al., 2011). Another study which investigated the reason for developmental delay, seizures and intellectual disability in members of a consanguineous family from Jordan identified a biallelic germline mutation in *CAMK2A*. This missense mutation caused a defective self-oligomerization of αCaMKII, thus preventing the assembly into a multimeric holoenzyme (Chia et al., 2018). Also mutations in other CaMKII subunit coding genes may result in intellectual disabilities (Proietti Onori et al., 2018). Overall, these findings support the view that human αCaMKII is involved in the development of many behavioural processes and that may become dysfunctional in mental disorders (Robison, 2014; Müller et al., 2016).

One of the major psychiatric disorders that shares molecular and anatomical pathways with learning and memory is drug addiction (Kelley, 2004; Hyman et al., 2006; Müller and Schumann, 2011). Specific associations of *CAMK2A* mutations with distinct features of drug addiction have been discovered (Müller et al., 2016). A human association study in alcohol

dependent people showed several associations of DSM-IV alcohol dependence with *CAMK2A* polymorphisms (Easton et al., 2013a). One of the seven associated SNPs in this study (rs10463293) has previously been associated with working memory performance (Easton et al., 2012). This would suggest an overlapping influence of *CAMK2A* genetic predisposition on learning/memory and addiction development. However, none of those SNPs overlapped with the SNP identified in the present study. In an association study in cocaine addicts from Brazil and Switzerland, none of the analyzed *CAMK2A* SNPs predicted whether a person was cocaine addict or not. However, within the population of the cocaine users, there was a significant association of SNP rs3776823 with addiction learning, i.e. the speed to establish severe cocaine consumption (Easton et al., 2014). Although rs3776823 is also an intronic SNP, it is localized only 425 bp upstream of exon 4 of the *CAMK2A* gene, which codes for the substrate binding pocket of the catalytic domain of αCaMKII. A recent study in Australian heroin addicts reported yet another *CAMK2A* association. Thereby, the minor allele of SNP rs10066581 (G-allele) was associated with a longer transition time from occasional to regular heroin use. This variant is located approximately 22 kb upstream of rs3776823 in intron 15 of the *CAMK2A* gene (Eirich et al., 2019). These findings suggest that numerous mutations and variants of *CAMK2A* which are either *de novo* or with already a certain population penetrance may not only alter learning and memory performance, but also contribute to the risk of psychiatric disorders where cognitive performance is just one of the symptom domains (Robison, 2014; Schumann et al., 2014; Müller et al., 2016).

A considerable limitation of this study was the unexpected, rather low population penetrance of the protective *CAMK2A* allele in both, the elderly and the adult population. This may at present stage prevent strong conclusions on associations and a potential mediation by morphological and cellular functional effects as previously described for other de novo mutations (Akira et al., 2018; Franzmeier et al., 2019). We only tested a population of elderly

patients with MCI or dementia, which show a higher population variance in memory performance than elderly people without this diagnosis. As such, it is unclear whether the association with *CAMK2A* may also apply to elderly people with normal cognitive performance. Thus, further studies with higher number of subjects in more distinct populations are warranted to confirm the initial evidence.

Taken together, the present study may provide first evidence for an association of a genetic variant of the *CAMK2A* gene specifically with retrieval of logical memory in elderly humans. This effect is possibly mediated by a higher hippocampal volume.

Conflict of interest

The authors declare no conflict of interest

Author statement

CPM, CR, CM, and BL conceived the study. TRS, AD, OP, AR, FJ, WM, MH, LF, ST, JW and JK gathered the samples and tested the subjects. CR, CM, GK, FB, AL, CPM and BL analyzed the data. CR, CM, BL, and CPM interpreted the experiments and wrote the first draft of the manuscript. All authors contributed to the final version of the manuscript.

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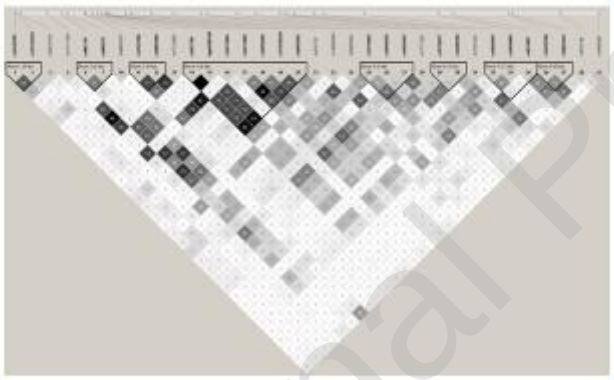

Figure 1

Figure 1 Variations of the *CAMK2A* gene according to University of California, Santa Cruz genome browser. Of the 42 SNPs included on the Illumina Omni-1M-Quad array, data of seven were excluded due to a MAF<0.05 and one due to a significant deviation from HWE ($p<0.05$). The figure illustrates the remaining 34 SNPs, five of which were excluded due to

LD ($R^2 \geq 0.8$). Altogether 29 SNPs were used to investigate relationships with the Wechsler memory scale (WMS) performance and hippocampal volumes. Haplotype blocks are based on confidence intervals. Numbers in squares denote pairwise R^2 between single markers. Gray scheme encodes LD between markers using R^2 confidence intervals (uninformative, light-gray and dark-gray; strong evidence for LD, black; strong evidence for recombination, white).

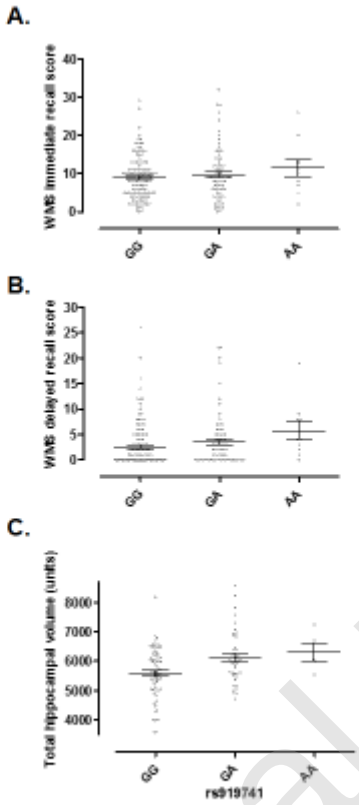

Figure 2

Figure 2 Associations of rs919741 *CAMK2A* SNP genotypes with logical memory and hippocampal volume in elderly people with cognitive impairments. While there was no effect of rs919741 on immediate recall of Wechsler memory scale (WMS) logical memory performance (A), the genotypes of the SNPs were significantly associated with delayed recall WMS performance (B) and total hippocampal volume (C). As compared to the major GG and GA genotypes, the minor AA genotype was related to a higher WMS delayed recall score

indicating better memory performance and a higher hippocampal volume. The graphs show mean and standard error of the mean.

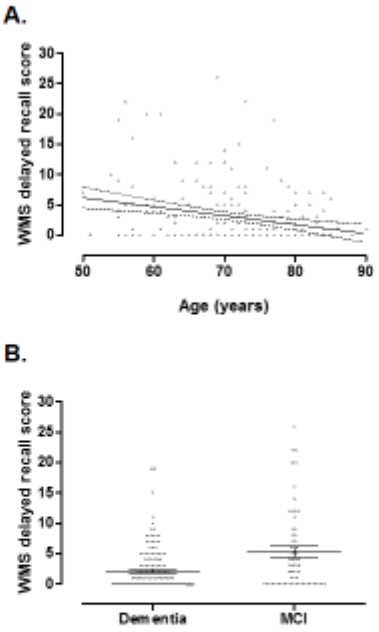

Figure 3

Figure 3 Associations between Wechsler memory scale (WMS) delayed recall score for logical memory and age and dementia vs. mild cognitive impairment (MCI) status. The WMS delayed recall score correlated negatively with age and was lower in patients with dementia than in subjects with MCI.

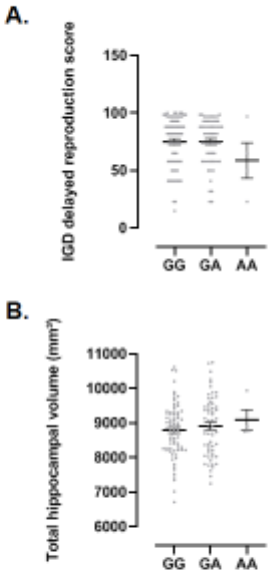

Figure 4

Figure 4 Associations of rs919741 *CAMK2A* SNP genotypes with logical memory and hippocampal volume in healthy young adults. There was no statistically significant effect of rs919741 on either delayed reproduction of a verbal task (scale A10 of the Inventar zur Gedächtnisdiagnostik, IGD) (A) or total hippocampal volume (B). The graphs show mean and standard error of the mean.

Table 1 *CAMK2A* SNPs and logical memory performance. The italicized *CAMK2A* SNPs reach nominal significance (p_a or p_b; linear regression modeling [ENTER method]), rs919741 is printed in bold because it is significantly related to WMS delayed recall performance after correction for multiple testing (Bonferroni, p_c). MAF minor allele frequency, HWE Hardy–Weinberg equilibrium, WMS Wechsler memory scale score, [1] 95% CI lower limit, [2] 95% CI upper limit, p_a corrected for age,

sex and mild cognitive impairment vs. dementia status, p_b additional correction using bootstrap (10,000 resamples), p_c p_a-values corrected according to Bonferroni, n.s. not significant.

Marker	Position on chromosome 5	Minor allele	Major allele	MAF	HWE	WMS immediate recall						WMS delayed recall					
					p	B	p_a	95%-CI[1,2]		p_b	p_c	B	p_a	95%-CI[1,2]		p_b	p_c
rs2240795	149589647	G	A	0.44	0.40	2.3	6.7E-01	-8.5	12.9	6.8E-01	n.s.	1.4	7.9E-01	-9.0	11.4	7.9E-01	n.s.
rs13153325	149590133	G	A	0.35	0.40	6.8	2.3E-01	-4.0	17.4	2.2E-01	n.s.	3.2	5.5E-01	-7.1	13.8	5.5E-01	n.s.
rs6579779	149590568	C	T	0.42	0.39	4.2	4.4E-01	-6.8	15.0	4.5E-01	n.s.	0.3	9.6E-01	-10.1	10.9	9.6E-01	n.s.
rs887347	149596241	A	G	0.39	0.22	-4.9	3.7E-01	-15.7	5.3	3.6E-01	n.s.	8.2	1.3E-01	-1.9	18.3	1.1E-01	n.s.
rs980272	149596955	T	C	0.24	0.97	6.0	3.7E-01	-6.4	17.1	3.2E-01	n.s.	*14.8*	*1.9E-02*	*2.8*	*25.7*	*1.1E-02*	*n.s.*
rs6882623	149599374	G	A	0.07	1.00	-16.8	1.3E-01	-39.9	4.3	1.4E-01	n.s.	-3.8	7.2E-01	-25.4	16.2	7.2E-01	n.s.
rs2163766	149599548	A	G	0.08	0.75	-9.5	3.5E-01	-30.3	10.0	3.6E-01	n.s.	-3.3	7.4E-01	-22.9	16.5	7.4E-01	n.s.
rs6869490	149600448	T	C	0.07	0.82	*-23.0*	*5.1E-02*	*-44.4*	*0.0*	*4.0E-02*	*n.s.*	-15.5	1.7E-01	-36.2	6.1	1.5E-01	n.s.
rs957709	149601826	C	T	0.35	0.29	3.9	4.9E-01	-7.3	14.8	4.9E-01	n.s.	*12.0*	*2.6E-02*	*1.8*	*22.0*	*2.0E-02*	*n.s.*
rs2241694	149602608	A	G	0.07	0.69	*-23.1*	*4.3E-02*	*-43.9*	*-1.6*	*3.1E-02*	*n.s.*	-9.4	3.9E-01	-28.8	11.1	3.5E-01	n.s.
rs2241695	149602824	C	T	0.43	0.08	-1.7	7.5E-01	-12.4	8.9	7.6E-01	n.s.	9.0	7.9E-02	-0.8	18.9	7.5E-02	n.s.
rs4958469	149605116	T	C	0.28	0.44	*11.2*	*6.2E-02*	*-0.4*	*22.0*	*4.8E-02*	*n.s.*	*16.4*	*4.3E-03*	*5.4*	*26.6*	*3.0E-03*	*n.s.*
rs919741	**149611688**	**A**	**G**	0.22	1.00	9.0	1.8E-01	-4.7	22.0	1.9E-01	n.s.	**21.7**	**7.0E-04**	**8.9**	**33.6**	**4.0E-04**	**<.05**
rs10066581	149615681	A	G	0.14	0.45	-9.8	2.5E-01	-25.6	5.3	2.2E-01	n.s.	-8.6	2.9E-01	-24.3	6.6	2.8E-01	n.s.
rs6869634	149616719	A	G	0.19	0.23	9.2	1.7E-01	-4.1	22.1	1.7E-01	n.s.	*17.6*	*6.0E-03*	*4.8*	*29.6*	*5.9E-03*	*n.s.*
rs3776825	149620671	T	C	0.30	1.00	5.6	3.6E-01	-6.0	16.7	3.4E-01	n.s.	8.5	1.5E-01	-2.9	19.5	1.4E-01	n.s.
rs4958456	149623365	A	G	0.13	0.95	-0.7	9.3E-01	-16.4	15.5	9.3E-01	n.s.	-4.0	6.2E-01	-19.4	12.1	6.2E-01	n.s.
rs4958454	149626752	C	T	0.44	0.47	-1.2	8.3E-01	-11.9	9.6	8.2E-01	n.s.	6.0	2.6E-01	-3.7	15.7	2.3E-01	n.s.
rs7711562	149627423	G	A	0.45	0.53	-0.4	9.4E-01	-11.5	10.3	9.4E-01	n.s.	4.5	3.9E-01	-5.4	14.4	3.7E-01	n.s.
rs3756577	149628644	A	G	0.14	0.94	0.0	1.0E+00	-16.3	15.9	1.0E+00	n.s.	-1.9	8.1E-01	-16.7	13.1	7.9E-01	n.s.
rs2288799	149631413	G	A	0.44	0.20	-0.2	9.7E-01	-10.7	10.1	9.7E-01	n.s.	1.4	7.9E-01	-8.5	11.5	7.8E-01	n.s.
rs7701427	149632955	T	C	0.18	0.96	5.3	4.8E-01	-8.3	19.0	4.5E-01	n.s.	3.8	5.9E-01	-10.6	18.5	6.2E-01	n.s.
rs4958445	149638360	T	C	0.27	0.58	-1.9	7.6E-01	-13.9	9.6	7.5E-01	n.s.	2.9	6.3E-01	-8.3	13.3	6.0E-01	n.s.
rs4958902	149648780	T	G	0.14	0.19	-5.0	5.1E-01	-18.9	9.3	4.8E-01	n.s.	0.3	9.7E-01	-13.1	14.3	9.6E-01	n.s.
rs874083	149650925	A	G	0.20	1.00	7.2	3.1E-01	-6.5	19.8	2.8E-01	n.s.	4.9	4.7E-01	-8.9	18.8	4.9E-01	n.s.
rs2228706	149658711	T	C	0.25	0.98	11.0	8.8E-02	-0.7	22.4	5.9E-02	n.s.	7.8	2.1E-01	-4.7	19.5	2.1E-01	n.s.
rs6894342	149659180	T	C	0.14	0.36	12.4	1.1E-01	-1.6	27.4	9.1E-02	n.s.	4.5	5.5E-01	-11.1	20.3	5.8E-01	n.s.
rs919740	149665849	C	T	0.24	0.94	-0.1	9.9E-01	-12.7	12.1	9.9E-01	n.s.	3.1	6.2E-01	-9.5	15.2	6.2E-01	n.s.
rs3806947	149671323	A	G	0.07	0.60	-10.4	3.6E-01	-31.1	11.1	3.2E-01	n.s.	5.8	5.9E-01	-13.1	25.0	5.4E-01	n.s.