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A Cross-sectional Study on the Association of KIBRA Genetic Polymorphism with Episodic Memory in North Jordanian Adults

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Abstract

BACKGROUND: Episodic memory (EM) is one of the cognitive processes most damaged by aging and is thought to be the system most susceptible to neurodegenerative illnesses. Recently, EM function has been linked to a single nucleotide polymorphism (rs17070145) in the ninth intron of the KIBRA gene (kidney and brain expressed gene).

AIM: This study looked at the relationship between the KIBRA polymorphism (rs17070145) and the EM abilities assessed by the Rey Auditory Verbal Learning Test and Rey Complex Figure Test at various time points (20-min, 30-min, 24-h, and 6-month).

METHODS: A total of 112 healthy adult Jordanians between the ages of 18 and 45 were included in the study, and the genotypes at the KIBRA (rs17070145) polymorphic site were identified using the PCR-RFLP method.

RESULTS: The outcomes did not reveal any statistically significant assessment of verbal and visual EM tests and the KIBRA polymorphism. The findings also indicated that KIBRA polymorphism had no statistically significant impact on short-term memory or learning capacity, indicating that KIBRA did not affect a person's ability to pay attention or concentrate. In the Jordanian population, the genotype percentages for KIBRA rs17070145 were: 10.7% for TT, 43.8% for TC, and 45.5% for CC, and the percentage of the T allele was 0.326.

CONCLUSION: The current investigation discovered no statistically significant differences between the Jordanian population and either the European or the South Asian populations in terms of the percentages of alleles and genotypes of the KIBRA rs17070145 gene.

Introduction

A crucial cognitive skill that allows people to encode, retain, and recall information is called memory [1], [2], [3]. It helps us read and have conversations while maintaining our interpersonal bonds. By unknowingly skimming through the experiences we have had in the past, our memories play a significant part in dictating how we behave in the present. In the past century, it has been proposed that memory is not just one brain function but a collection of numerous distinct systems connected-non-conscious memory recall of techniques for carrying out different jobs and skills.

Declarative memory refers to memories that are instantly available to conscious remembrance. I deals with knowledge gained through learning, such as facts, data, and experience [4].

They proposed three distinct systems as possible divisions of memory, namely, the memory mechanism known as sensory memory (SM) records information from all senses using an exact copy of the experience. Short-Term Memory (STM): STM has a limited capacity for information storage and is used to retain any information attended from SM. The Stage Model's last storage facility, the Long-Term Memory (LTM), has an infinite capacity. What we mistakenly think of as forgetting is a retrieval failure. LTM is similarly categorized into many systems as STM. LTM can be categorized in several ways, including non-declarative (implicit) and declarative (explicit) forms [4]. The definition of non-declarative memory, often known as procedural memory or implicit memory, is a non-conscious memory recall of techniques for carrying out different jobs and skills. On the other hand, declarative memory refers to memories that are instantly available to conscious remembrance. It deals with knowledge gained via learning, such as facts, data, and experiences [4].

Declarative memory has been split into episodic and semantic memory (memory for general knowledge). Episodic memory (EM) is the conscious recall of facts or events, i.e., the recall of directly experienced events [5]. Episodental memory stands out from the other memory systems mentioned above since it is tied to our experiences and related to our emotions. EM is the only memory system that enables us to go back in time and connect the past to the present.

Unexpectedly, several researches suggested that EM might also be involved in imagining the future [6].

The most vulnerable part of neurocognitive memory to neuropsychiatric disorders and degenerative conditions, including Alzheimer's disease and Huntington's disease, is classified as EM [7], [8].

Studies on identical and fraternal twins have demonstrated that human memory is a polygenic cognitive trait with an estimated heritability of 50%, which suggests that genetic variability significantly impacts this fundamental brain cognitive ability [9], [10], [11]. In the first genome-wide association study (GWAS) to identify memory-related genetic variation, an intronic polymorphism (rs17070145) in the gene encoding kidney and brain-expressed protein (KIBRA) was associated with delayed recall performance [12].

The gene that codes for KIBRA, known as WWC1 (also known as WW and C2 domain containing 1), is found on the sense strand of chromosome 5q34. It has 26 exons and encodes a protein mostly produced in the kidney and the brain [13].

Two WW domains are present in human KIBRA (amino acids 6–86); each domain is made up of around 40 amino acids and features two tryptophan residues that are highly conserved at the N-terminus. Proteins having proline-rich motifs, such as PPxY, are recognized by WW domains (where x represents any amino acid and Y represents tyrosine). In addition, KIBRA has a C2 domain (amino acids 655–783), i.e., home to two four-stranded sheets that interact with phospholipids in a way that is Ca2+-sensitive [14].

The KIBRA gene's ninth intron has the common TC substitution rs1707045 (GenBank accession number NM 015238). They demonstrated that in cognitively normal cohorts from Switzerland and the United States, T allele carriers performed better on verbal and visual EM tasks than C/C carriers. Furthermore, the hippocampus, a crucial part of the brain associated with memory function, was also found to be much more activated in non-carriers of the T allele. Other research also confirmed the enhanced EM performance in T allele carriers [15], [16], [17], [18], [19], [20], [21], [22], [23]. Moreover, reduced KIBRA level was found to mediate memory loss and synaptic plasticity decline from a functional standpoint [24].

However, according to four research [25], [26], [27], [28], there is no correlation between KIBRA gene polymorphism and EM performance, and one study even found that T-allele carriers perform worse [29]. Furthermore, numerous investigations revealed a strong connection between the T-allele and lowering the risk of getting Alzheimer's disease [30], [31], [32]. In addition, a different study verified the link between the C-allele of the rs17070145 gene and an elevated risk of Alzheimer's [23].

Following the identification and characterization of the KIBRA protein, most studies on KIBRA have been on its function in neurological diseases and cognitive processes.

Another research provided evidence that an intronic polymorphism (rs17070145) of the KIBRA gene is associated with delayed recall performance in three cognitively normal cohorts from Switzerland and the United States. This was the first GWAS to identify memory-related genetic variation [12]. In addition, a different study supported the effect of the KIBRA rs17070145 T-allele on enhanced cognitive performance. It provided evidence for the 1st time linking the aforementioned genetic variation to spatial mental ability in humans [23].

In the brain, there was a high expression of the KIBRA protein, particularly in regions connected to memory, including the hippocampus [12]. KIBRA is primarily located in the perinuclear area and the postsynaptic density of neurons [33]. By interacting with other proteins, the KIBRA protein is involved in several cellular processes, including cell polarity, vesicle trafficking, and synaptogenesis. Three postsynaptic proteins, dendrin, synaptopodin, and Protein Kinase C (PKC), are among the at least ten direct interaction partners of the KIBRA protein; PKC has been proposed as the protein most strongly associated with KIBRA's functional role in memory [34], [35].

Numerous studies suggest that KIBRA plays a crucial part in how humans form memories. In addition, numerous investigations revealed a strong connection between the T-allele and lowering the risk of getting Alzheimer's disease [30], [31], [32].

The correlation between KIBRA polymorphism and EM function in healthy subjects has been replicated in several investigations. To the best of our knowledge, most later studies, including healthy participants, have successfully replicated the link between T allele carriers and superior memory function [15], [16], [17], [18], [19], [20], [21], [36], [37]. On the other hand, according to four research [25], [26], [27], [28], there is no connection between KIBRA polymorphism and memory performance.

Interestingly, only one study, which looked at a sample of people with subjective memory complaints, has found a connection between having the T allele (TT and TC genotypes) and performing poorly on LTM tests [29]. In light of that, for a more profound knowledge of our memory and to develop novel treatment options that can combat cognitive impairments like Alzheimer's disease, it is crucial to study genes that affect memory function. Since KIBRA polymorphism has not yet been investigated about EM performance in the Jordanian population, the current study's goal was to look into any potential relationships between the KIBRA gene's single-nucleotide polymorphism (rs17070145) and verbal-visual EM performance, as well as immediate memory and learning capacity, in healthy adult Jordanians. In addition, it analyzes how the Jordanian population's KIBRA genotypes are distributed.

Materials and Methods

Human subject descriptions and blood sample collection

This study was approved by Institutional Review Board (IRB) Committee at the King Abdullah University Hospital (Ref 75/117/2018. August 8, 2018, Irbid, Jordan) and Yarmouk University (Ref 13/1/23034. August 12, 2018, Irbid, Jordan). A total of 112 healthy adults were recruited in this study. The study sample was composed of 44 females and 68 males (mean age= 27.8 ± 7.6 years, age range=18–45). They were recruited by randomly distributing sign-up sheets to the undergraduate and postgraduate students and employees at Yarmouk University/Irbid-Jordan. An appointment was made with those who wished to participate by telephone, and each person was interviewed separately. Two neuropsychological assessments (RAVLT and ROCFT) were applied to any participant who met the inclusion criteria. In addition, the inclusion criteria included the following: (1) had an Arabic mother tongue, (2) had at least 12 years of formal education, (3) had normal hearing and vision (4) right-handedness. In contrast, any participant who had any of the following features were excluded from the study: (1) a history of neurological or psychiatric disorders, (2) received any psychiatric medication which affects the cognitive ability, (3) a history of cancer, (4) head injury with loss of consciousness (5) any type of cardiovascular diseases (6) any substance dependence like alcohol or drugs (7) epilepsy or seizures (8) first-or second-degree relatives with Alzheimer disease (9) diabetes with either type I or type II.

Sample collection: Three milliliters of venous blood were collected from each participant in an Ethylenediamine tetraacetic acid tube and stored at 4°C until further use.

Molecular analysis

DNA was extracted from blood samples using G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology Company, Gyeonggi, South Korea), according to the manufacturer's protocol. A spectrophotometric analysis was performed to determine the concentration and purity of the extracted DNA. All measurements were taken via Epoch™/Take3™ instrument (BioTek, Winooski, USA). To check the integrity of the extracted DNA, all extracted samples were subjected to agarose gel electrophoresis on 1% agarose gel (stained with RedSafe™ staining solution, 20,000×) in 1× TBE buffer with 1 Kb ready-to-use DNA Ladder (GeneDireX, Taoyuan, Taiwan) at 80 volts for 1 h. Two primers were used to amplify a 198 base-pair fragment containing the polymorphic site rs17070145 [26]. The PCR amplification was carried out by Aeris™ Thermal Cycler (ESCO, Changi, Singapore) in a total reaction volume of 50 μL and the two primers (Forward: 5'atcctcttgaggcttcactgg3', the reverse: 3'actttcaacacaatgaacaagg5') was used. To verify the validity of the PCR procedure, we subjected the PCR products to agarose gel electrophoresis on 3% agarose gel (stained with RedSafe™ staining solution, 20,000×) in 0.5X TBE buffer with 50 bp DNA marker (ZYMO Research) at 75 Volts for 70 min.

Restriction digestion was performed in a total reaction volume of 23.5 μL. Briefly, 20 μL of PCR products were digested by the addition of 1.5 μL (15 Units) of MseI restriction enzyme (New England Biolabs, Ipswich, MA, USA) and 2 μL of 10× enzyme buffer (New England Biolabs, Ipswich, MA, USA) then, the reaction mixture was incubated in Aeris™ Thermal Cycler (ESCO, Changi, Singapore) at 37°C for 20 h. The resulting restriction products were analyzed using electrophoresis on 3% agarose gel (stained with RedSafe) in 1× TBE buffer. A 50 bp molecular weight marker (ZYMO Research) was used to assess the size of restriction fragments. The gel was visualized by UV-T and photographed. Because the MseI restriction site is present in T-allele and is absent in C-allele, the restriction fragments give three possibilities: two bands (128 and 70 bp) which indicates a homozygous TT genotype, one band (198 bp), which indicates a homozygous CC genotype and three bands (198, 128, and 70 bp) which indicates a heterozygous TC genotype.

Statistical analysis

The analysis of data was performed in two stages. In the first stage, the differences between the RAVLT and ROCFT measures (including EM indices) were compared across the three KIBRA genotype groups: heterozygous TC, homozygous TT, and homozygous CC. In the second stage and due to the low frequency of TT genotype carriers (n = 12), the TT and TC genotypes were combined in one group (T-allele carriers) and compared the mean differences of the RAVLT and ROCFT against the CC genotype group (non-T-allele carriers). Dummy numbers were given for previous genotype groups before the analysis (1 = TC, 2 = TT, 3 = CC, 4 = TC+TT). The two genders were also given dummy numbers (0 = Males, 1 = Female) before analysis.

The differences between the KIBRA genotype groups in regards to demographic variables (age, prior years of education, and sex) were assessed using one-way Analysis of Variances (ANOVA) for the three genotype groups (TC, TT, CC) and by t-test for the two genotype groups (CC and TC+TT).

To search for any effect of the KIBRA genotype on attention, working memory, and motivation (confounding factors), the mean differences of the following scores of RAVLT and ROCFT: trial 1 (immediate recall for list A), trial 6 (immediate recall for list B), trial 7 (recalling list A immediately after recalling list B), total immediate recall trials (Trial 1+ Trial 6+ Trial 7),

and the copy trial of ROCFT across the different KIBRA genotype groups were investigated. To assess the effect of genotype on learning, the mean difference of total learning score of the RAVLT (Trials 1+ 2+ 3+ 4+ 5) across genotype groups was tested.

The mean scores of the RAVLT and ROCFT were compared across the three genotype groups using one-way ANOVA, and Leven's test tested the homogeneity of variance. Student's t-tests for independent samples were used to compare the mean scores for the CC genotype group against the T-allele carrier group (TC+TC).

Statistical significance was defined at $p < 0.05$, and all p-values reported were two-tailed. All analyses were done using IBM Statistical Package for the Social Sciences.

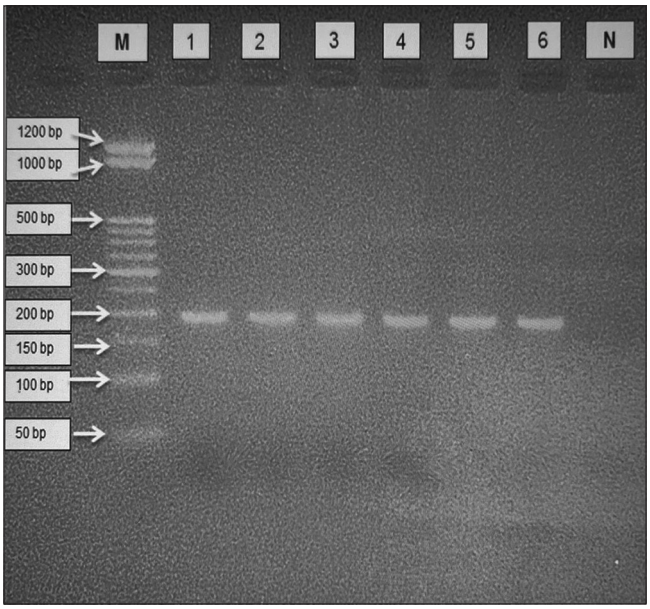

Figure 1: PCR products were separated by gel electrophoresis on 3% agarose. The figure shows clear bands with 198 bp size for each of the six samples. M= 50 bp DNA marker (ZYMO Research), Lanes 1 - 6 = bands of 198 bp amplified DNA fragments, N= negative control, which contained all PCR reaction components except the DNA template

Results and Discussion

A 112 healthy adult participants with a mean age of 27.81 years old (age range: 18–45 years) were enrolled in this study. Sixty-one percent were male (n = 68), and 39% were female (n = 44). The mean of prior years of education for all participants was 16.3 years. All participants were Jordanian, had Arabic mother tongue, and met the inclusion criteria for this study.

The genomic DNA was successfully extracted from all samples, and the integrity was assessed by gel electrophoresis. The fragment of 198 bp, which contains the polymorphic site rs17070145, was successfully amplified using two primers (F: atcctcttgaggcttcactgg and R: actttcaacacaatgaacaagg). Figure 1 shows the PCR amplification products of some representative samples.

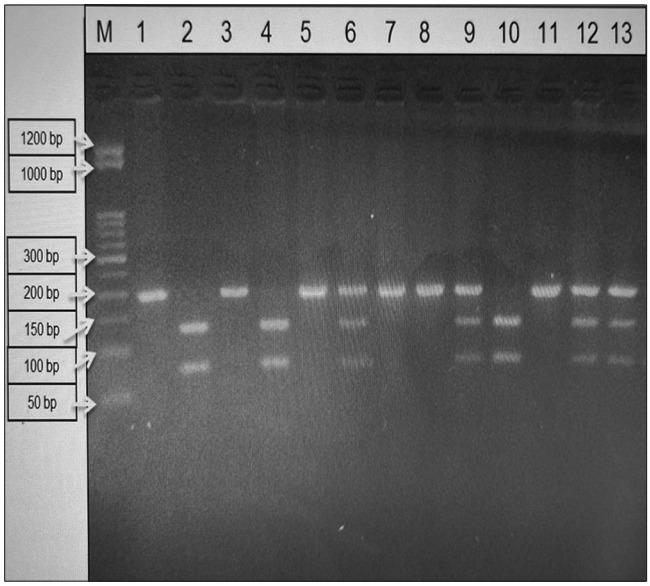

Figure 2: Representative gel for determination of KIBRA rs17070145 polymorphism. The figure shows three band patterns. Lanes 1, 3, 5, 7, 8, and 11 represent CC genotypes (one 198 bp band for each lane); Lanes 6, 9, 12, and 13 represent TC genotypes (three bands for each lane with 198, 128, and 70 bp in size), Lanes 2, 4, and 10 represent TT genotypes (two bands for each lane with 128 and 70 bp in size); M = 50 bp DNA marker (ZYMO Research)

Figure 2 shows the digestion products of the three different genotypes found in the samples.

The current study analysis showed that 12 participants were genotyped as TT carriers (10.7 %), 49 as TC carriers (43.8 %), and 51 as CC carriers (45.5 %). The observed T and C alleles' frequencies were 0.326 and 0.674, respectively. Figure 3 shows the percentages of KIBRA genotypes among the Jordanian population according to the current study.

We first categorized all participants into three genotype groups (the heterozygous TC group vs. the homozygous TT group vs. the homozygous CC group). There were no statistically significant differences in age ($p = 0.65$) (Table 1), education years ($p = 0.61$) (Table 2), and gender ($p = 0.92$) Figure 3 between the three KIBRA genotype groups. See also (Table 3) for a summary of the three groups' comparisons regarding demographic variables.

Table 1: The difference between the three *KIBRA* genotype groups regarding prior years of education

Genotype	n	Mean prior years of education	Standard Deviation	95% Confidence interval for mean		F	p-value
				Lower Bound	Upper Bound		
TC	49	16.47	1.58	16.01	16.92		
TT	12	16.33	2.39	14.82	17.85	0.50	0.61
CC	51	16.04	2.58	15.31	16.76		
Total	112	16.26	2.16	15.85	16.66		

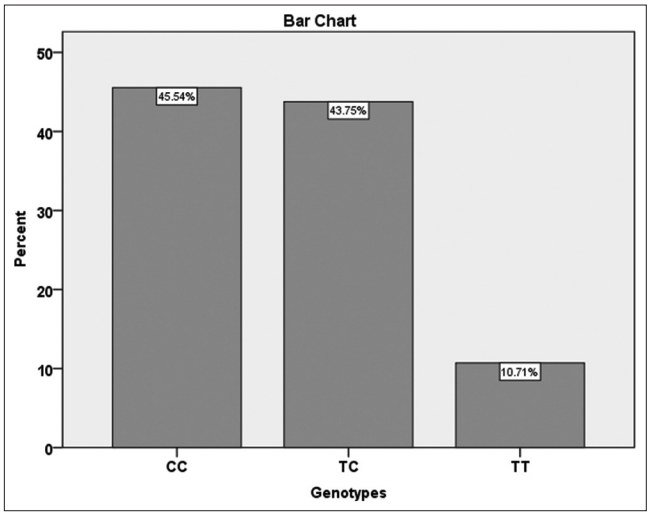

Figure 3: The percentage of KIBRA Genotypes among the Jordanian population

Due to the low frequency of the TT genotype (10.7%), we combined TT and TC genotype groups into one group (TC/TT) and tested their differences against the CC genotype group of KIBRA rs17070145 (CC vs. TC/TT). The previous step of combining TT and TC genotype groups was done by the original study [12] and the subsequent studies [15], [16], [17], [18], [20], [21], [26], [28], [29].

Table 2: Summary of demographic characteristics of participants according to the three *KIBRA* rs17070145 genotype groups

Demographic /Genotype groups	TC (n = 49)	TT (n = 12)	CC (n = 51)
Age (mean years) (95% CI)	27.16 (24.97, 29.36)	29.25 (24.41, 34.09)	28.10 (26.02, 30.18)
Sex ratio (Males: Females)	29:20	7:5	32:19
Prior years of education mean (95% CI)	16.47 (16.02,16.92)	16.33 (14.82, 17.85)	16.04 (15.32, 16.76)

It is seen that the distribution of genotypes for any gender group is nearly the same, which indicates a weak or no association between the two variables. The Pearson Chi-square test of independence for these data is found insignificant (p = 0.921), which assures this conclusion.

Table 3: Demographic characteristics of participants according to the two *KIBRA* rs17070145 genotype groups (TC/TT vs. CC)

Demographic /Genotype groups	TC/TT (n = 6 1)	CC (n = 51)
Age (years) (SD)	27.57 (7.62)	28.10 (7.39)
Sex ratio (Males: Females)	36:25	32:19
Mean prior years of education. (SD)	16.44 (1.75)	16.04 (2.58)

It is seen that the distribution of the two Genotype groups (TC/TT and CC) for either gender group is nearly the same, which indicates a weak or no association between the two variables. The Pearson Chi-square test of independence for these data is found insignificant (p = 0.687), which assures this conclusion (Figures 4 and 5).

To search for any effect of KIBRA genotypes groups on attention, working memory, and concentration, we investigated the mean differences of the following scores of RAVLT and ROCFT: Trial 1 (immediate recall for list A), Trial 6 (immediate recall for list B), Trial 7 (recalling list A immediately after recalling list B), Total immediate recall trials (Trial 1+ Trial 6+ Trial 7), and the Copy trial of ROCFT across the different KIBRA genotype groups. To assess the effect of genotype on learning ability, we test the mean difference of total learning score of the RAVLT (Trials 1+ 2+ 3+ 4+ 5) across genotype groups.

Table 4: Immediate recall and learning performance across the three *KIBRA* rs17070145 genotype groups (TC vs. TT vs. CC)

Measure	TC (n = 49) M (SD)	TT (n = 12) M (SD)	CC (n = 51) M (SD)	p-value
RAVLT Trial 1	8.84 (2.02)	8.75 (0.97)	8.80 (1.87)	0.99
RAVLT Trial 6	8.35 (2.44)	8.75 (1.29)	8.10 (1.79)	0.59
RAVLT Trial 7	13.04 (1.78)	13.00 (2.13)	13.20 (1.50)	0.88
RAVLT Total immediate recalls (Trials 1+6+7)	30.22 (5.10)	30.50 (3.58)	30.10 (3.70)	0.96
RAVLT Total learning (Trials 1+2+3+4+5)	60.94 (6.53)	60.92 (6.13)	61.84 (6.28)	0.75
RCFT copy trial	32.99 (2.21)	32.67 (1.50)	33.06 (2.71)	0.88

M: Mean, SD: Standard deviation, RAVLT: Rey auditory verbal learning test.

There were no significant differences in any measure of immediate recall and learning between KIBRA rs17070145 genotype groups. Table 4 shows a comparison of TC versus TT versus CC, and Table 5 shows a comparison of TC/TT versus CC.

Table 5: Immediate recall and learning performance between *KIBRA* T- carriers and non-carriers

Measure	TC/TT (n = 61) M (SD)	CC (n = 51) M (SD)	p-value
RAVLT Trial 1	8.82 (1.86)	8.80 (1.87)	0.97
RAVLT Trial 6	8.43 (2.25)	8.10 (1.79)	0.40
RAVLT Trial 7	13.03 (1.83)	13.20 (1.50)	0.61
RAVLT Total immediate recalls (Trials 1+6+7)	30.28 (4.81)	30.10 (3.70)	0.83
RAVLT Total learning (Trials 1+2 + 3+4+5)	60.93 (6.40)	61.84 (6.28)	0.45
RCFT copy trial	32.93 (2.09)	33.06 (2.71)	0.77

M: Mean, SD: Standard deviation, RAVLT: Rey auditory verbal learning test, RCFT: Rey complex figure test. Statistical significance was defined at p < 0.05, and all p values reported are two-tailed.

Differences in Verbal EM between KIBRA genotype groups were assessed by different mean indices of RAVLT. These mean indices are Trial 8 (delayed recall after 20 minutes), Trial 9 (recognition), Trial 10 (delayed recall after 24 h), and Trial 11 (delayed recall after 6 months). Further, the mean difference of the total verbal EM was assessed by adding the previous indices (Trials 8+ 9 + 10+ 11) together. There were no significant differences between the three KIBRA genotype groups (TC vs. TT vs. CC) in any previous measure of verbal EM (p > 0.05) (Table 6). There were also no significant differences between the two KIBRA genotype groups (TT/TC vs. CC) (p > 0.05; Table 7 and Figure 6).

Table 6: Verbal episodic memory performance between the three *KIRBRA* genotype groups

Measure	TC (n = 49) M (SD)	TT (n = 12) M (SD)	CC (n = 51) M (SD)	p-value
RAVLT Trial 8 (20-min delayed recall)	13.04 (1.78)	12.5 (2.39)	13.22 (1.58)	0.45
RAVLT Trial 9 (recognition)	14.67 (0.69)	14.5 (1.17)	14.75 (0.52)	0.53
RAVLT Trial 10 (24-h delayed recall)	12.55 (1.86)	12.25 (1.91)	12.31 (1.68)	0.76
RAVLT Trial 11 (6-month delayed recall)	4.90 (2.87)	5.08 (3.23)	4.59 (3.06)	0.82
RAVLT Total verbal Episodic Memory (Trials 8+9+10+11)	45.16 (5.15)	44.33 (6.57)	44.75 (5.10)	0.86

M: Mean, SD; Standard deviation, RAVLT: Rey auditory verbal learning test, Statistical significance was defined at p < 0.05, and all p values reported are two-tailed.

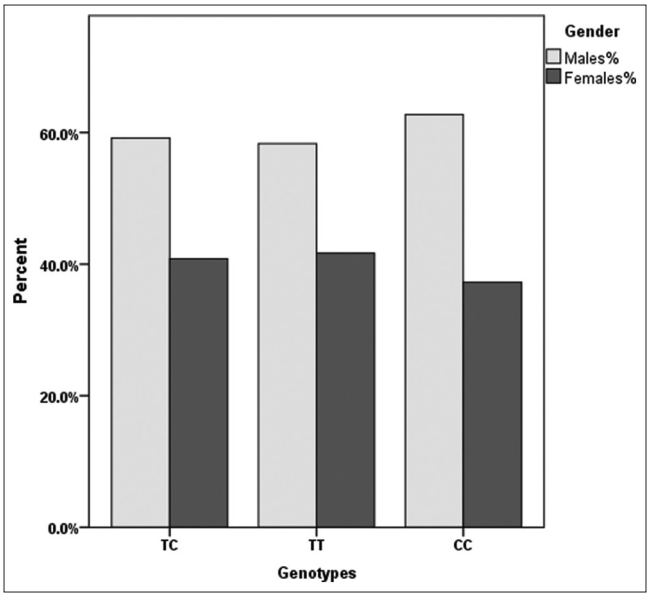

Figure 4: Clustered bar chart for gender and genotypes (TC vs. TT vs. CC)

Further, Two-way ANOVA was conducted to investigate if there was an interaction effect between gender and genotype on total verbal EM. No significant interaction effect between gender and genotype on total verbal EM was present (p = 0.11; Table 8).

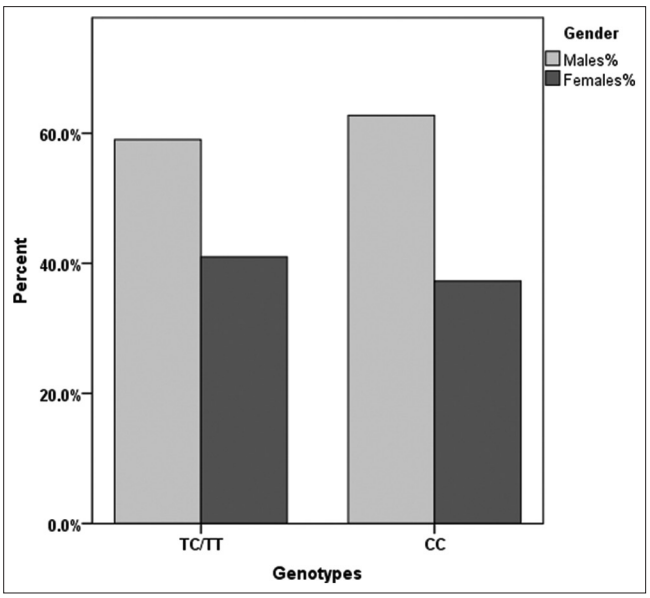

Figure 5: Clustered bar chart for Gender and Genotypes (TC/TC vs. CC)

Visual EM performance

Performance on the RCFT was assessed using mean recall after a 3-min trial, mean recall after a 30-min trial, percentage retention for a 3-min trial, percentage retention for a 30-min trial, and finally, the sum of 3-min and 30-min recall scores. Percentage retention was defined as the ratio of delayed recall score (3 min, 30 min) relative to the copy score (RCFT recall score/RCFT copy score) × 100) (Table 9).

Table 7: Comparison of verbal episodic memory performance between *KIBRA* T allele carriers and non-carriers

Measure	TC/TT (n = 61) M (SD)	CC (n = 51) M (SD)	p-value
RAVLT Trial 8 (20-min delayed recall)	12.93 (1.90)	13.22 (1.58)	0.40
RAVLT Trial 9 (recognition)	14.64 (0.80)	14.75 (0.52)	0.42
RAVLT Trial 10 (24-h delayed recall)	12.49 (1.86)	12.31 (1.68)	0.60
RAVLT Trial 11 (6-month delayed recall)	4.93 (2.91)	4.59 (3.06)	0.54
RAVLT Total Episodic Memory (Trials 8+9+10+11)	45 (5.40)	44.75 (5.10)	0.80

M: Mean, SD: Standard deviation, RAVLT: Rey auditory verbal learning test, Statistical significance was defined at p < 0.05, and all p values reported are two-tailed.

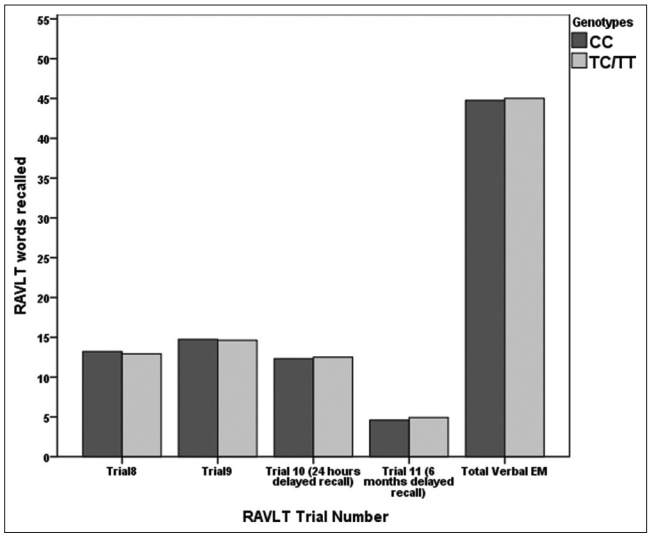

Figure 6: Clustered bar chart for genotypes ((TC/TC Vs.CC) and verbal EM indices (RAVLT Trials 8, 9, 10, 11 and their sum abbreviated as total verbal EM) It is seen that the mean scores of the RAVLT trials for either genotype group ((TC/TT and CC) are nearly the same, which indicates no association between the two variables (all p values > 0.05). RAVLT: Rey auditory verbal learning test, EM: Episodic memory, Trial 8: 20 min delayed recall, Trial 9: Recognition trial

Table 8: Interaction effect of *KIBRA* genotypes and gender on total verbal episodic memory

Genotypes	Gender	Mean	SD	n	p-value
TC	Males	43.93	5.36	29	0.11
	Females	46.95	4.35	20	
TT	Males	41.00	5.83	7	
	Females	49.00	4.58	5	
CC	Males	44.38	5.11	32	
	Females	45.37	5.17	19	

Statistical significance was defined at p < 0.05, and the p value reported is two-tailed.

Table 9: Visual episodic memory performance between the three *KIRBRA* genotype groups

Measure	TC (n = 49) M (SD)	TT (n = 12) M (SD)	CC (n = 51) M (SD)	p-value
RCFT 3-min recall	23.73 (4.50)	22.17 (4.45)	23.21 (4.76)	0.56
RCFT 30 min recall	23.61 (4.54)	22.63 (3.50)	22.96 (5.14)	0.71
3 min Percentage retention	72.00 (13.03)	67.83 (12.40)	70.04 (12.65)	0.54
30 min percentage retention	71.76 (13.67)	69.33 (9.95)	69.18 (13.61)	0.61
RCFT 3 min+30 min recall scores	47.35 (8.86)	44.79 (7.82)	46.17 (9.73)	0.64

M: Mean, SD: Standard deviation, RCFT: Rey complex figure test, Statistical significance was defined at p < 0.05, and all p values reported are two-tailed.

No significant differences between KIBRA genotype groups on any previous measures of visual EM were present (p > 0.05; Table 10).

Two-Way ANOVA was conducted to investigate if there was an interaction effect between gender and genotype on total visual EM (3 min+ 30 min delayed recall scores). The analysis showed no significant interaction effect between gender and genotype on total visual EM (p > 0.05; Table 11).

Table 10: Visual episodic memory performance between the two *KIRBRA* genotype groups (T carriers vs. non-carriers)

Measure	TC/TT (n = 61) M (SD)	CC (n = 51) M (SD)	p-value
RCFT 3-min delayed recall	23.43 (4.49)	23.21 (4.76)	0.80
RCFT 30 min delayed recall	23.42 (4.35)	22.96 (5.14)	0.61
3 min Percentage retention	71.18 (12.92)	70.04 (12.65)	0.64
30 min percentage retention	71.28 (12.99)	69.18 (13.61)	0.41
RCFT 3 min+30-min recall scores	46.84 (8.67)	46.17 (9.73)	0.70

M: Mean, SD: Standard deviation, RCFT: Rey complex figure test, Statistical significance was defined at $p < 0.05$, and all p values reported are two-tailed.

The primary objective of this study was to determine how the KIBRA (WWC1) rs17070145 polymorphism affected Jordanian adults' verbal and visual EM abilities. The three KIBRA genotype groups performed similarly for verbal and visual EM. Our findings showed that T allele carriers of the KIBRA gene (combined TT and TC genotypes) did not significantly outperform non-carriers on the RAVLT and RCFT tests (CC genotype).

Table 11: Interaction effect of *KIBRA* genotype and gender on total visual episodic memory

Genotypes	Gender	Mean	SD	n	p-value
TC	Males	47.74	9.67	29	0.60
	Females	46.78	7.75	20	
TT	males	42.64	5.91	7	
	female	47.80	9.81	5	
CC	males	46.06	10.37	32	
	females	46.34	8.83	19	

Statistical significance was defined at $p < 0.05$, and the p value reported is two-tailed.

In this study, various RAVLT and RCFT measures were used to evaluate EM. According to our knowledge, the current study is the only one to have looked at the relationship between KIBRA polymorphism and EM after a delayed interval of 6 months (in addition to 20 min and 24 h), which might help explain why EM tests are becoming more sensitive.

Our results diverge from prior findings [12], which employed RAVLT and delayed memory of 5 min and 24 h for terms without any semantic link (30 min delayed recall). They found that T-allele carriers outperformed non-carriers in verbal EM by a significant margin. Our findings are clearly at odds with some later studies that made use of RAVLT and showed that the T allele has a favourable impact on verbal EM performance [16], [17], [21].

The current study's findings did not support the claim made in earlier research that used neuropsychological tests other than RAVLT that the T allele positively impacts verbal EM capacity. For instance, the item-paired memory task was used in the prior study [18], and the Wechsler Memory Scale-Revised (WMS-R) was used to assess EM in other studies [19], [20], [37]. All the researchers above found that the T allele positively affected verbal EM function.

Our findings support prior studies that revealed no connection between the T allele and verbal EM performance. Three earlier studies that also employed RAVLT and found no correlation between the T allele of the KIBRA gene (rs17070145) and verbal memory performance exist in addition to the current study [25], [26], [28]. The T allele had no effect, according to one study that employed CERAD [27]. Interestingly, a previous investigation [29] found that T-allele carriers performed much worse on verbal EM tests than SMC persons with CC genotypes (TT and TC). The KIBRA genotypes may have varied effects on memory performance in participants who reported memory problems than those who did not. These contradicting findings may be the consequence of variations in the methods used to evaluate EM function, which may have influenced the outcomes of genetic association studies [29].

Similar to earlier research [19], [20], [26], [27], [28], [29], this investigation was unable to identify any correlation between KIBRA polymorphism and visual EM ability ($p > 0.05$). In addition to the current investigation, it should be noted that all previous studies that failed to discover an association had evaluated visual EM using standardized tests, such as the WMS-R, CERAD, or the RCFT [26], [27], [28], [29]. The previous study [12] is the only one to date that has documented a link between KIBRA and T allele carriers performing better than non-carriers in terms of visual memory. According to another researcher [18], the utilized unstandardized test may have contributed to the positive relationship found in the other finding [12].

Additionally, the findings of this study did not reveal any appreciable variations in immediate or learning measurements between KIBRA genotype groups, supporting the vast majority of other studies' findings that the KIBRA SNP has no effects on frontal lobe functions such as attention, motivation, and concentration [12], [16], [26], [28], [29], [37]. But few studies have found a link between the KIBRA SNP and attention [15], [20]. These erratic results could be attributed to variations in memory tests and ethnic groups [20].

The fact that KIBRA and other brain gene SNPs may interact to alter EM performance may explain why this investigation was unable to detect any correlation between KIBRA polymorphism and EM. Several genes may influence human EM. This assertion was corroborated by the earlier investigation [18], which discovered that the presence of the CLSTN2 C-allele, which codes for the synaptic protein calsyntenin 2, increased the positive effects of the KIBRA T-allele. Other investigations observed no significant correlation between the allele status of either SNP (KIBRA and CLSTN2) and any memory test, contradicting this claim [27].

Remembering that the current study enlisted healthy adult volunteers is important (age range: 18–45 years). In two investigations, a connection between the KIBRA polymorphism and EM in adults and middle-aged people was successfully demonstrated [12], [18]. Other researchers contended that older individuals would be more likely to show this link (higher effect sizes) than younger individuals [16]. This shows that age is a key factor in genetic research that looks at EM function.

Table 12: Comparison of T allele percentage of *KIBRA* rs17070145 between Jordanian population and other populations

Population	Sample size	T allele percentage	Z-value	p-value	Is there a statistical difference?
Jordanians (Current study)	112	0.326	-	-	-
African	1322	0.613	−5.93	<0.00001	Yes
East Asian	1008	0.765	−9.83	<0.00001	Yes
European	1006	0.354	−0.59	0.56	No
South Asian	978	0.29	0.79	0.43	No
Vietnamese	214	0.72	−6.86	<0.00001	Yes
American	694	0.56	−4.60	<0.00001	Yes

However, other research found that only included adults aged 75–76 revealed no connection between the KIBRA polymorphism and EM function. This is obviously at odds with the other findings [27].

The subjects' vitamin concentrations were not measured in either the current trial or the earlier KIBRA experiments. Although very little evidence supports the link between vitamin B12 and memory [38], the participants' vitamin intake likely conceals the impact of KIBRA genotypes on memory function.

These often contradicting findings show that it is difficult to research memory, a fundamental cognitive skill in humans. Therefore, to unravel the enigma of our brain, we must make more attempts to discover fresh approaches and methods.

The study's secondary goal was to find the genotype distribution and allelic frequency for KIBRA rs17070145 in the Jordanian population. The genotypes of 12 participants were found to be 10.7% TT carriers, 43.8% TC carriers, and 45.5% CC carriers. The frequencies of the detected T and C alleles were 0.326 and 0.674, respectively. The National Center for Biotechnology Information reports that the KIBRA rs17070145 allelic frequency varies significantly amongst populations. There was no statistically significant difference in the percentage of T-alleles between the populations of Europe (0.354) and South Asia (0.29), and Jordan (0.326). In contrast, we found a statistically significant difference in the percentage of T-alleles in the African (0.613), East Asian (0.765), American (0.56), and Vietnamese populations compared to Jordanians (0.326) and Vietnamese (0.72) populations (Table 12).

Conclusion

In conclusion, KIBRA polymorphisms do not affect the EM phenotype. This investigation builds on earlier research that used two of the most widely used neuropsychology tests to assess the impact of the KIBRA rs17070145 polymorphism on EM (RAVLT and RCFT). We believe that this is the first study to look at how the KIBRA polymorphism affects EM in the Middle Eastern population, specifically in healthy adult Jordanians. We recommend that prospective KIBRA study participants get their vitamin levels checked, especially their B12 levels. Additionally, whereas others hypothesized that KIBRA genotypes significantly impact EM function, which allows detection even in a small sample, we advise verifying our work with a bigger sample in other Jordanian locations [16].

References

1. Goldstein G. Neuropsychological assessment of memory. In: Neuropsychology (Human Brain Function: Assessment and Rehabilitation). New York, USA: Springer; 1998. p. 334-52.
2. Schwab LC, Luo V, Clarke CL, Nathan PJ. Effects of the KIBRA single nucleotide polymorphism on synaptic plasticity and memory: A review of the literature. Curr Neuropharmacol. 2014;12(3):281-8. https://doi.org/10.2174/1570159X11666140104001553
   PMid:24851092
3. Sherwood L. Human Physiology: From Cells to Systems. 9th ed. Boston, USA: Cengage Learning; 2016. p. 157.
4. Squire LR. Memory systems of the brain: A brief history and current perspective. Neurobiol Learn Mem. 2004;82(3):171-7. https://doi.org/10.1016/j.nlm.2004.06.005
   PMid:15464402
5. Tulving E. Episodic and Semantic Memory. New York, USA: Academic Press; 1972.
6. Hassabis D, Kumaran D, Vann SD, Maguire EA. Patients with hippocampal amnesia cannot imagine new experiences. Proc Natl Acad Sci U S A. 2007;104(5):1726-31. https://doi.org/10.1073/pnas.0610561104
   PMid:17229836
7. Nyberg L, McIntosh AR, Tulving E. Functional brain imaging of episodic and semantic memory with positron emission tomography. J Mol Med (Berl). 1998;76(1):48-53. https://doi.org/10.1007/s001090050189
   PMid:9462867
8. Tulving E, Markowitsch HJ. Episodic and declarative memory: Role of the hippocampus. Hippocampus. 1998;8(3):198-204. https://doi.org/10.1002/(SICI)1098-1063(1998)8:3<198:AID-HIPO2>3.0.CO;2-G
   PMid:9662134
9. McClearn GE, Johansson B, Berg S, Pedersen NL, Ahern F, Petrill SA, *et al*. Substantial genetic influence on cognitive abilities in twins 80 or more years old. Science. 1997;276(5318):1560-3. https://doi.org/10.1126/science.276.5318.1560
   PMid:9171059
10. Bouchard TJ Jr., McGue M. Genetic and environmental influences on human psychological differences. J Neurobiol. 2003;54(1):4-45. https://doi.org/10.1002/neu.10160
    PMid:12486697
11. Volk HE, McDermott KB, Roediger HL 3rd, Todd RD. Genetic influences on free and cued recall in long-term memory tasks. Twin Res Hum Genet. 2006;9(5):623-31. https://doi.org/10.1375/183242706778553462
    PMid:17032541
12. Papassotiropoulos A, Stephan DA, Huentelman MJ, Hoerndli FJ, Craig DW, Pearson JV, *et al*. Common Kibra alleles are associated with human memory performance. Science. 2006;314(5798):475-8. https://doi.org/10.1126/

science.1129837
PMid:17053149

13. Kremerskothen J, Plaas C, Büther K, Finger I, Veltel S, Matanis T, *et al*. Characterization of KIBRA, a novel WW domain-containing protein. Biochem Biophys Res Commun. 2003;300(4):862-7. https://doi.org/10.1016/s0006-291x(02)02945-5
PMid:12559952
14. Duning K, Wennmann DO, Bokemeyer A, Reissner C, Wersching H, Thomas C, *et al*. Common exonic missense variants in the C_2 domain of the human KIBRA protein modify lipid binding and cognitive performance. Transl Psychiatry. 2013;3(6):e272. https://doi.org/10.1038/tp.2013.49
PMid:23778582
15. Almeida OP, Schwab SG, Lautenschlager NT, Morar B, Greenop KR, Flicker L, *et al*. KIBRA genetic polymorphism influences episodic memory in later life, but does not increase the risk of mild cognitive impairment. J Cell Mol Med. 2008;12(5A):1672-6. https://doi.org/10.1111/j.1582-4934.2008.00229.x
PMid:18194457
16. Schaper K, Kolsch H, Popp J, Wagner M, Jessen F. KIBRA gene variants are associated with episodic memory in healthy elderly. Neurobiol Aging. 2008;29(7):1123-5. https://doi.org/10.1016/j.neurobiolaging.2007.02.001
PMid:17353070
17. Bates TC, Price JF, Harris SE, Marioni RE, Fowkes GF, Stewart MC, *et al*. Association of KIBRA and memory. Neurosci Lett. 2009;458(3):140-3. https://doi.org/10.1016/j.neulet.2009.04.050
PMid:19397951
18. Preuschhof C, Heekeren HR, Li SC, Sander T, Lindenberger U, Backman L. KIBRA and CLSTN2 polymorphisms exert interactive effects on human episodic memory. Neuropsychologia. 2010;48(2):402-8. https://doi.org/10.1016/j.neuropsychologia.2009.09.031
PMid:19804789
19. Vassos E, Bramon E, Picchioni M, Walshe M, Filbey FM, Kravariti E, *et al*. Evidence of association of KIBRA genotype with episodic memory in families of psychotic patients and controls. J Psychiatr Res. 2010;44(12):795-8. https://doi.org/10.1016/j.jpsychires.2010.01.012
PMid:20185150
20. Yasuda Y, Hashimoto R, Ohi K, Fukumoto M, Takamura H, like N, *et al*. Association study of KIBRA gene with memory performance in a Japanese population. World J Biol Psychiatry. 2010;11(7):852-7. https://doi.org/10.3109/15622971003797258
PMid:20509760
21. Witte AV, Köbe T, Kerti L, Rujescu D, Flöel A. Impact of KIBRA polymorphism on memory function and the hippocampus in older adults. Neuropsychopharmacology. 2016;41(3):781-90. https://doi.org/10.1038/npp.2015.203
PMid:26156558
22. Porter T, Burnham SC, Doré V, Savage G, Bourgeat P, Begemann K, *et al*. KIBRA is associated with accelerated cognitive decline and hippocampal atrophy in APOE ε4-positive cognitively normal adults with high Aβ-amyloid burden. Sci Rep. 2018;8(1):2034. https://doi.org/10.1038/s41598-018-20513-y
PMid:29391469
23. Kazantseva AV, Enikeeva RF, Davydova YD, Mustafin RN, Takhirova ZR, Malykh SB, *et al*. The role of the KIBRA and APOE genes in developing spatial abilities in humans. Vavilovskii Zhurnal Genet Selektsii. 2021;25(8):839-46. https://doi.org/10.18699/VJ21.097
PMid:35088019
24. Heitz FD, Farinelli M, Mohanna S, Kahn M, Duning K, Frey MC, *et al*. The memory gene KIBRA is a bidirectional regulator of synaptic and structural plasticity in the adult brain. Neurobiol Learn Mem. 2016;135:100-14. https://doi.org/10.1016/j.nlm.2016.07.028
PMid:27498008
25. Need AC, Attix DK, McEvoy JM, Cirulli ET, Linney KN, Wagoner AP, *et al*. Failure to replicate effect of Kibra on human memory in two large cohorts of European origin. Am J Med Genet B Neuropsychiatr Genet. 2008;147B(5):667-8. https://doi.org/10.1002/ajmg.b.30658
PMid:18205171
26. Wersching H, Guske K, Hasenkamp S, Hagedorn C, Schiwek S, Jansen S, *et al*. Impact of common KIBRA allele on human cognitive functions. Neuropsychopharmacology. 2011;36(6):1296-304. https://doi.org/10.1038/npp.2011.16
PMid:21346737
27. Sédille-Mostafaie N, Sebesta C, Huber KR, Zehetmayer S, Jungwirth S, Tragl KH, *et al*. The role of memory-related gene polymorphisms, KIBRA and $CLSTN_2$, on replicate memory assessment in the elderly. J Neural Transm (Vienna). 2012;119(1):77-80. https://doi.org/10.1007/s00702-011-0667-9
PMid:21643791
28. Franks KH, Summers MJ, Vickers JC. KIBRA gene polymorphism has no association with verbal or visual episodic memory performance. Front Aging Neurosci. 2014;6:270. https://doi.org/10.3389/fnagi.2014.00270
PMid:25339899
29. Nacmias B, Bessi V, Bagnoli S, Tedde A, Cellini E, Piccini C, *et al*. KIBRA gene variants are associated with episodic memory performance in subjective memory complaints. Neurosci Lett. 2008;436(2):145-7. https://doi.org/10.1016/j.neulet.2008.03.008
PMid:18378080
30. Corneveaux JJ, Liang WS, Reiman EM, Webster JA, Myers AJ, Zismann VL, *et al*. Evidence for an association between KIBRA and late-onset Alzheimer's disease. Neurobiol Aging. 2010;31(6):901-9. https://doi.org/10.1016/j.neurobiolaging.2008.07.014
PMid:18789830
31. Burgess JD, Pedraza O, Graff-Radford NR, Hirpa M, Zou F, Miles R, *et al*. Association of common KIBRA variants with episodic memory and AD risk. Neurobiol Aging. 2011;32(3):557.el-9. https://doi.org/10.1016/j.neurobiolaging.2010.11.004
PMid:21185624
32. Wang HF, Tan L, Yu JT, Ma XY, Liu QY, Wang W. Age-dependent association of KIBRA gene polymorphism with Alzheimer's disease in Han Chinese. Mol Biol Rep. 2013;40(12):7077-82. https://doi.org/10.1007/s11033-013-2830-x
PMid:24190487
33. Johannsen S, Duning K, Pavenstädt H, Kremerskothen J, Boeckers TM. Temporal-spatial expression and novel biochemical properties of the memory-related protein KIBRA. Neuroscience. 2008;155(4):1165-73. https://doi.org/10.1016/j.neuroscience.2008.06.054
PMid:18672031
34. Büther K, Plaas C, Barnekow A, Kremerskothen J. KIBRA is a novel substrate for protein kinase Czeta. Biochem Biophys Res Commun. 2004;317(3):703-7. https://doi.org/10.1016/j.bbrc.2004.03.107
PMid:15081397
35. Schneider A, Huentelman MJ, Kremerskothen J, Duning K, Spoelgen R, Nikolich K. KIBRA: A new gateway to learning and memory? Front Aging Neurosci. 2010;2:4. https://doi.org/10.3389/neuro.24.004.2010
PMid:20552044

36. Kauppi K, Nilsson LG, Adolfsson R, Eriksson E, Nyberg L. KIBRA polymorphism is related to enhanced memory and elevated hippocampal processing. J Neurosci. 2011;31(40):14218-22. https://doi.org/10.1523/jneurosci.3292-11.2011
PMid:21976506

37. Muse J, Emery M, Sambataro F, Lemaitre H, Tan HY, Chen Q, *et al*. WWC1 genotype modulates age-related decline in episodic memory function across the adult life span. Biol Psychiatry. 2014;75(9):693-700. https://doi.org/10.1016/j.biopsych.2013.09.036
PMid:24290728

38. Health Quality Ontario. Vitamin B_{12} and cognitive function: An evidence-based analysis. Ont Health Technol Assess Ser. 2013;13(23):1-45.
PMid:24379897