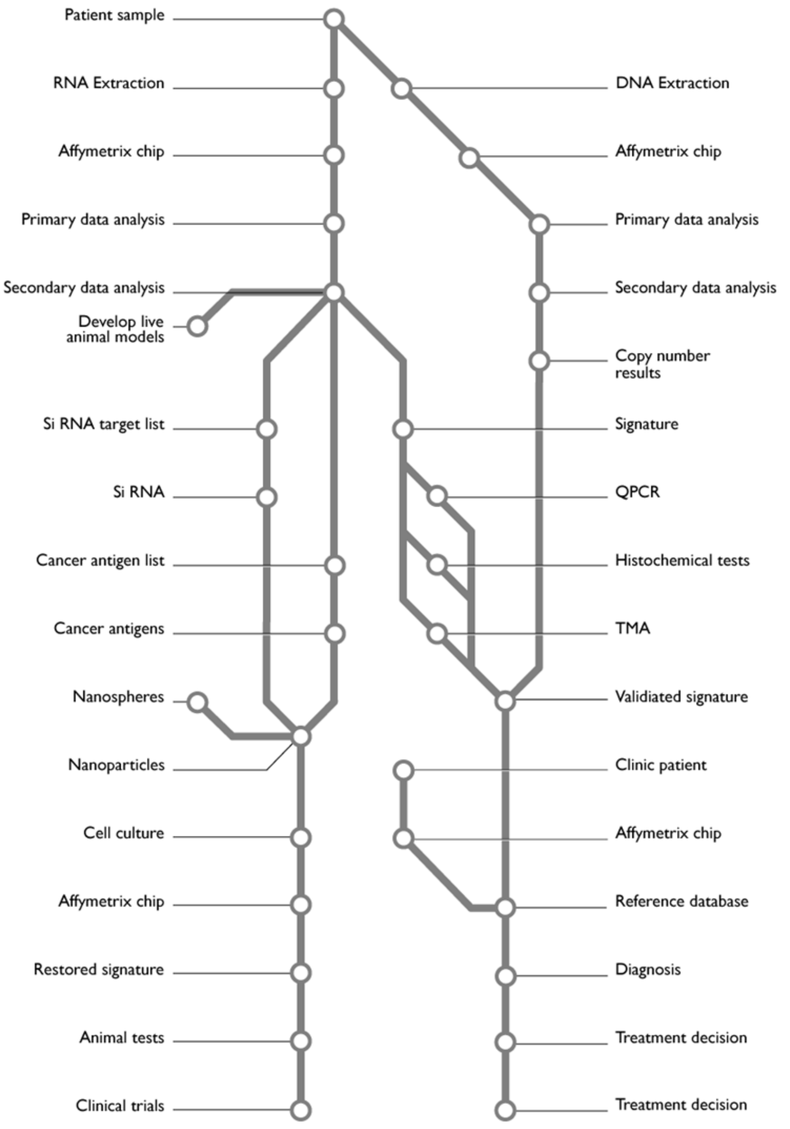
Patient sample
RNA Extraction
Affymetrix chip
Primary data analysis
Secondary data analysis
Develop live animal models
Si RNA target list
Si RNA
Cancer antigen list
Cancer antigens
Nanospheres
Nanoparticles
Cell culture
Affymetrix chip
Restored signature
Animal tests
Clinical trials
DNA Extraction
Affymetrix chip
Primary data analysis
Secondary data analysis
Copy number results
Signature
QPCR
Histochemical tests
TMA
Validiated signature
Clinic patient
Affymetrix chip
Reference database
Diagnosis
Treatment decision
Treatment decision

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Barreca D, Gasparotto A, Maragno C, Tondello E.

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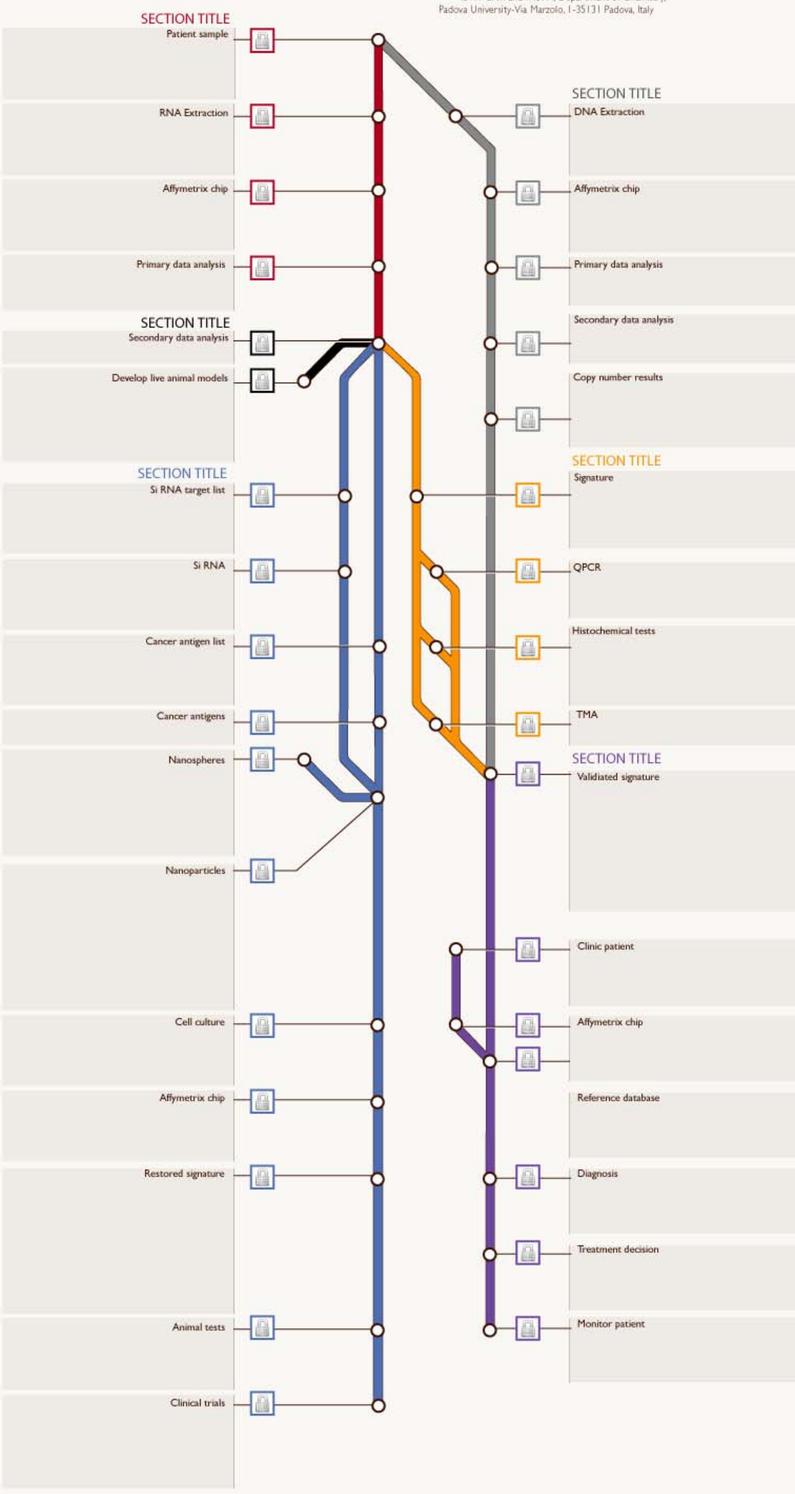


COMMON KIBRA ALLELES DETERMINE MEMORY PERFORMANCE IN HUMANS

Papassotiropoulos A, Stephan DA, Huentelman MJ, Frederic J, Hoerndli FA, Craig DW, Pearson JV, Huynh KD, Corneveaux J, Osborne D, Mondadori C Buchmann A, Reiman EM, Caselli RJ, Henke K, de Quervain DJF. Common KIBRA alleles determine memory performance in humans. Science. 2006 X;Y(Z):xxxx-xx.

POPULATION ANALYSIS

Swiss population

351 healthy young Swiss subjects (240 females, 111 males; mean age 22.8 ± 0.2 [standard error] years). Complete description of study explained to subjects, written informed consent obtained. Ethics committee of the Canton of Zurich, Switzerland approved study protocol.

DNA Extraction

Genetic Heterogeneity Analysis

Individually genotyped 351 Swiss subjects at 318 unlinked SNPs using Pyrosequencing method (ABI)

Structured Population Analysis

Analyzed genotypes using STRUCTURE software, estimating subject ancestry with a priori assumptions of either K = 2, 3, 4, 5 and 6 discrete subpopulations. Found moderate allele- frequency divergence. Participants' genetic backgrounds formed one normally distributed cluster (P=0.6). Ten subjects identified as outliers (i.e. probability of cluster allocation lower than 25%) and excluded from the genetic association studies.

WHOLE-GENOME MEMORY ASSOCIATION: GENOTYPING

Verbal Memory Test

Subjects viewed 6 series of 5 semantically unrelated nouns presented at a rate of 1 word per second with the instruction to learn the words for immediate free recall after each series. Subjects underwent an unexpected delayed free-recall test of the learned words after 5 min and again after 24 h. Both delayed recall tests reflect episodic memory (11). 24-hr recall additionally requires long-term synaptic changes (29). Switzerland approved study protocol.

Stratified Population into Four Phenotypes

Remaining population (n=341) stratified into 4 groups based on 5-min free recall performance (i.e. bottom 25%, bottom 50%, top 50%, and top 25% performers).

DNA Quantitation & Pooling

Individual genomic DNA concentrations of the subjects determined using the Quant-iT® PicoGreen/Æ dsDNA Assay Kit (Invitrogen). Each individual contributed a total of 120 ng of DNA to the pool and each pool was created de novo a total of three times.

500K Microarray Processing

Each of 4 pools diluted to 50 ng/ l with reduced TE buffer and genotyped at 502,627 SNPs as described in Early Access v 2.0 of Mendel Array protocol (Affymetrix). Labeled DNA hybridized onto respective Mendel array at 49∞C for 18 hours at 60 rpm. Each hybridized array was washed, stained, and scanned according to the manufacturer's (Affymetrix) instructions.

WHOLE-GENOME MEMORY ASSOCIATION: DATA ANALYSIS

Memory Association Statistics

500K SNP allelic frequencies were based on corresponding Relative Allele Signal (RAS) scores calculated with custom PERL script (freely available at www.tgen.com). Poor performing SNPs were cropped.

Discovered Two Associated SNPs

Two SNPs found significant with both analysis strategies: rs17070145 and rs6439886. Both SNPs map within genes expressed in the human brain: rs17070145 is a common T /C substitution within the ninth intron of KIBRA (encoding the neuronal protein KIBRA), rs6439886 is a common T/C substitution within the first intron of CLSTN2 (encoding the synaptic protein calsyntenin 2).

ASSOCIATION VALIDATION (US)

SNP Validation in New Population (US)

Both SNPs evaluated in independent population of 256 cognitively normal older participants from the United States. (171 females, 85 males; mean age 54.0 ± 0.7 [standard error] years).

Individual Genotyping (US)

Genotyping of SNPs rs17070145 (KIBRA) and rs6439886 (CLSTN2) in the US samples done by PyrosequencingTM (Uppsala, Sweden) on a PSQ 96 MA machine.

Memory Testing of US Population

The Auditory Verbal Learning Test (AVLT) (12) and the Buschke's Selective Reminding Test (SRT) (13) used to quantify verbal episodic memory. Executive functions, attention and working memory quantified by the Wisconsin Card Sorting Test and the Paced Auditory Serial Attention Task (13).

SNP/Memory Association (US)

Validated KIBRA SNP association with differential human memory performance (5-min and 24-h recall). CLS2 SNP did not validate.

	Immediately recalled words (mean ± s.e)	Words recalled after 5 min (mean ± s.e)	Words recalled after 24 h (mean ± s.e)
rs17070145[a]			
C/C, n = 164	23.6 ± 0.3	7.6 ± 0.2[c]	6.7 ± 0.2[b]
C/T & T/T, n = 169	24.1 ± 0.3	9.4 ± 0.2[c]	8.0 ± 0.2[b]
rs6439886			
T/T, n = 265	23.9 ± 0.2	8.4 ± 0.2[c]	7.3 ± 0.2[d]
T/C & C/C, n = 76	24.2 ± 0.4	9.8 ± 0.4[c]	8.4 ± 0.4

ASSOCIATION VALIDATION (SWISS)

Individual Genotyping (Swiss)

Genotyping of SNPs rs17070145 (KIBRA) and rs6439886 (CLSTN2) in the Swiss samples done by PyrosequencingTM (Uppsala, Sweden) on a PSQ 96 MA machine.

SNP/Memory Association (Swiss)

Validated both KIBRA and CLSTN2 SNP associations with differential human memory performance (5-min and 24-h recall).

	Immediately recalled words (AVLT) (mean ± s.e)	Words recalled after 30 min (AVLT) (mean ± s.e)	Free recall of words (SRT) (mean ± s.e)
rs17070145			
C/C, n = 126	9.4 ± 0.3	8.5 ± 0.3[a]	83.7 ± 1.3[b]
C/T & T/T, n = 130	10.0 ± 0.3	9.7 ± 0.3[a]	90.3 ± 1.1[b]
rs6439886[a]			
T/T, n = 185	9.7 ± 0.2	9.1 ± 0.2	88.4 ± 0.9
T/C & C/C, n = 64	9.9 ± 0.4	9.2 ± 0.4	88.9 ± 1.6

KIBRA SNP Association With Episodic Memory Validated

HYPOTHESIS TESTING

Tested KIBRA Activity in Hippocampus

15 Swiss carriers of the KIBRA T-allele and 15 non-carriers tested by functional MRI. Allelic groups matched for sex (5 males and 10 females in each group), education (P = 0.7), age (P = 0.8), the His452Tyr genotype of the 5-HT2a receptor gene (2) (P = 0.4), and for 5-min delayed recall performance (P = 1.0). Impact of the KIBRA genotype on hippocampal activation tested through face-profession associative task.

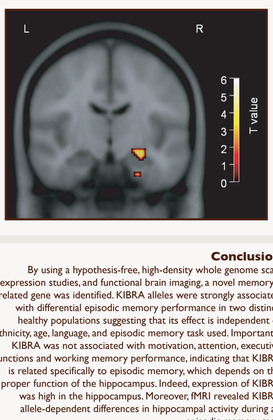
Expression of KIBRA in the Brain

Expression levels of KIBRA determined in the human brain: hippocampus, the frontal and parietal lobe. RT-PCR amplicons designed detecting both KIBRA full-length transcript and its truncated form KIAA0869, which lacks the first 223 amino acids and which is likely formed through an alternate transcriptional start site (16).

KIBRA Active in Hippocampus

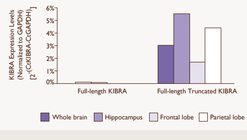
Non-carriers of the T allele showed increased activity in medial temporal lobe, frontal cortex and parietal cortex. In addition to the hippocampus, also these neocortical regions belong to a network important for episodic memory retrieval (17).

Non-carriers of the T allele need more activation in memory retrieval-related brain regions to reach the same level of retrieval performance as T allele carriers. During memory encoding, no allele-dependent differences in brain activations were found, suggesting that the genotype did not affect episodic memory at this early stage of memory formation. In an additional working memory task, no allele-dependent differences in brain activation in these regions were seen, indicating that the above reported activations in non-carriers were specific to episodic memory retrieval. Automated voxel-based algorithms (SPM2) (22) and manual volume measurements failed to reveal significant allele-dependent differences in the hippocampus or the parahippocampus (P = 0.1), suggesting that imaging results were not biased by morphological differences.



Truncated KIBRA Expressed in the Brain

Expression levels of full-length KIBRA in the human brain were marginal. In contrast, expression levels of truncated KIBRA high in all brain regions examined, with highest levels in the hippocampus. Importantly, SNP rs17070145 maps within the truncated form of KIBRA. KIBRA expression patterns in the human brain are consistent with a role in memory performance.

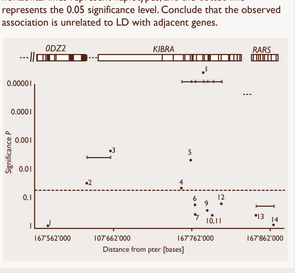


Tested LD Around KIBRA Locus

14 common SNPs used to fine-map region harboring KIBRA, RARS, and part of ODZ2. Used Amplifluor genotyping system and PowerMarker Version 3.22 to assess linkage disequilibrium and construct haplotypes. Multifactorial analyses of covariance done for simultaneous assessment of the influence of age, sex, education, and genotype effects on cognitive test performance. All tests were two-tailed.

KIBRA Not in LD

High levels of linkage disequilibrium (P < 0.001) detected between SNPs 2 and 3, between SNPs 4 and 12, and between SNPs 13 and 14. SNP8 and the corresponding haplotype yielded highest significance levels (P = 0.000004 and P = 0.000008, respectively). Dots represent SNPs, continuous horizontal lines represent haplotypes, and the dotted line represents the 0.05 significance level. Conclude that the observed association is unrelated to LD with adjacent genes.



Conclusion

By using a hypothesis-free, high-density whole genome scan, expression studies, and functional brain imaging, a novel memory-related gene was identified. KIBRA alleles were strongly associated with differential episodic memory performance in two distinct, healthy populations suggesting that its effect is independent of ethnicity, age, language, and episodic memory task used. Importantly, KIBRA was not associated with motivation, attention, executive functions and working memory performance, indicating that KIBRA is related specifically to episodic memory, which depends on the proper function of the hippocampus. Indeed, expression of KIBRA was high in the hippocampus. Moreover, fMRI revealed KIBRA allele-dependent differences in hippocampal activity during an episodic memory task.