

Common *Kibra* Alleles Are Associated with Human Memory Performance

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Human memory is a polygenic trait. We performed a genome-wide screen to identify memory-related gene variants. A genomic locus encoding the brain protein *KIBRA* was significantly associated with memory performance in three independent, cognitively normal cohorts from Switzerland and the United States. Gene expression studies showed that *KIBRA* was expressed in memory-related brain structures. Functional magnetic resonance imaging detected *KIBRA* allele-dependent differences in hippocampal activations during memory retrieval. Evidence from these experiments suggests a role for *KIBRA* in human memory.

Human memory is a polygenic cognitive trait. Heritability estimates of ~50% suggest that naturally occurring genetic variability has an important impact on this fundamental brain function (1). Recent candidate-gene association studies have identified some genetic variations with significant impact on human memory capacity (2–5). However, the success of these studies depends upon preexisting information, which limits their potential to identify unrecognized genes and molecular pathways (6, 7).

Recent advances in the development of high-density genotyping platforms now allow for high-resolution whole-genome association studies for polygenic phenotypes (8, 9). We used a genome-wide screen with more than 500,000 single-nucleotide polymorphisms (SNPs). The initial screen was done in pooled DNA from a Swiss cohort that was stratified into groups of participants with different levels of episodic memory performance (verbal delayed recall). SNPs fulfilling the selection criteria of the pooling stage (10) were genotyped individually in this same cohort and validated in two additional cohorts from the United States and from Switzerland.

We recruited 351 young adults (median age, 22 years; range, 18 to 48 years) from Switzerland (Swiss cohort 1) (10). Genetic association studies in outbred populations such as this one may be prone to false positives because nonrandom genetic heter-

ogeneity within the study sample (population structure) can lead to spurious associations between a genetic marker and a phenotype (11). Therefore, we controlled for genetic background and found no evidence of significant population stratification; the participants' genetic backgrounds formed one normally distributed cluster ($P = 0.6$) (10, 12). We identified 10 participants as outliers (probability of cluster allocation lower than 25%) and excluded them from the genetic association studies. The remaining population ($n = 341$) was stratified into four groups according to their performance in a verbal memory task which quantified the retrieval success 5 min after learning a word list comprising 30 semantically unrelated nouns (10). Each of these groups was genotyped at 502,627 SNPs. To minimize the possibility of false positives (at the apparent cost of false negatives), we combined two different and stringent statistical approaches (a single-point method and, as a filter for the most significant SNPs, a sliding-window method) to select SNPs of

Table 1. Association of SNPs rs17070145 (*KIBRA*) and rs6439886 (*CLSTN2*) with verbal episodic memory in Swiss cohort 1. Genotype calls of eight subjects were not considered for analysis due to low-quality pyrograms for rs17070145. Means with common superscripts are significantly different according to multifactorial analysis of variance. Data are means \pm SEM.

	<i>n</i>	No. of words recalled		
		Immediately	After 5 min	After 24 hours
rs17070145				
CC	164	23.6 \pm 0.3	7.6 \pm 0.2*	6.7 \pm 0.2†
CT/TT	169	24.1 \pm 0.3	9.4 \pm 0.2*	8.0 \pm 0.2†
rs6439886				
TT	265	23.9 \pm 0.2	8.4 \pm 0.2‡	7.3 \pm 0.2§
TC/CC	76	24.2 \pm 0.4	9.8 \pm 0.4‡	8.4 \pm 0.4§

* $P = 0.000004$ † $P = 0.0008$ ‡ $P = 0.002$ § $P = 0.022$

high statistical confidence (10). Two SNPs fulfilled these selection criteria and were prioritized for subsequent individual genotyping to exclude pooling-related false positives: rs17070145 and rs6439886. Both SNPs map within genes expressed in the human brain: rs17070145 is a common T \rightarrow C substitution within the ninth intron of *KIBRA* (GenBank accession number NM_015238), encoding a neuronal protein, and rs6439886 is a common T \rightarrow C substitution within the first intron of *CLSTN2* (encoding the synaptic protein calysntenin 2) (NM_022131).

Both the *KIBRA* and *CLSTN2* SNPs were also significantly associated with differential human memory performance when we genotyped them individually in Swiss cohort 1 using an independent genotyping technology (10). Carriers of *KIBRA* rs17070145 T allele had 24% better free recall performance 5 min after word presentation ($P = 0.000004$) and 19% better free recall performance 24 hours after word presentation ($P = 0.0008$) than did noncarriers (Table 1, table S1, and fig. S2). TT and CT genotype groups of rs17070145 were combined because the frequency of the TT genotype was low and because both groups displayed similar memory performance (table S1). SNP rs6439886 yielded similar results; however, the mean difference of memory performance between genotype groups was lower than that of rs17070145 (Table 1 and table S1). Both the 5-min and the 24-hour delayed free recall reflected episodic, hippocampus-dependent memory (13). Neither SNP was associated with performance on immediate recall tests (Table 1 and table S1), indicating that the allele-dependent differences in episodic memory were not caused by allelic effects on con-

Table 2. Association of SNPs rs17070145 (*KIBRA*) and rs6439886 (*CLSTN2*) with episodic memory in the U.S. population. The SRT was completed by 200 participants (98 CC carriers and 102 CT and TT carriers of rs17070145). Genotype calls of seven participants were not considered for analysis because of low-quality pyrograms for rs6439886. Means with common superscripts are significantly different according to multifactorial analysis of variance. Data are means \pm SEM.

	<i>n</i>	No. of items recalled		
		Immediately (AVLT)	After 30 min (AVLT)	Free recall task (SRT)
rs17070145				
CC	126	9.4 \pm 0.3	8.5 \pm 0.3*	83.7 \pm 1.2†
CT/TT	130	10.0 \pm 0.3	9.7 \pm 0.3*	90.3 \pm 1.1†
rs6439886				
TT	185	9.7 \pm 0.2	9.1 \pm 0.2	88.4 \pm 0.9
TC/CC	64	9.9 \pm 0.4	9.2 \pm 0.4	88.9 \pm 1.6

* $P = 0.004$ † $P = 0.00005$

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foundings factors such as motivation, attention, or working memory.

Both SNPs were further evaluated in a second, independent population of 256 cognitively normal participants (median age, 55 years; range, 20 to 81 years) from the United

Table 3. Association of SNP rs17070145 (*KIBRA*) with visual episodic memory in Swiss cohort 2. Data are means \pm SEM.

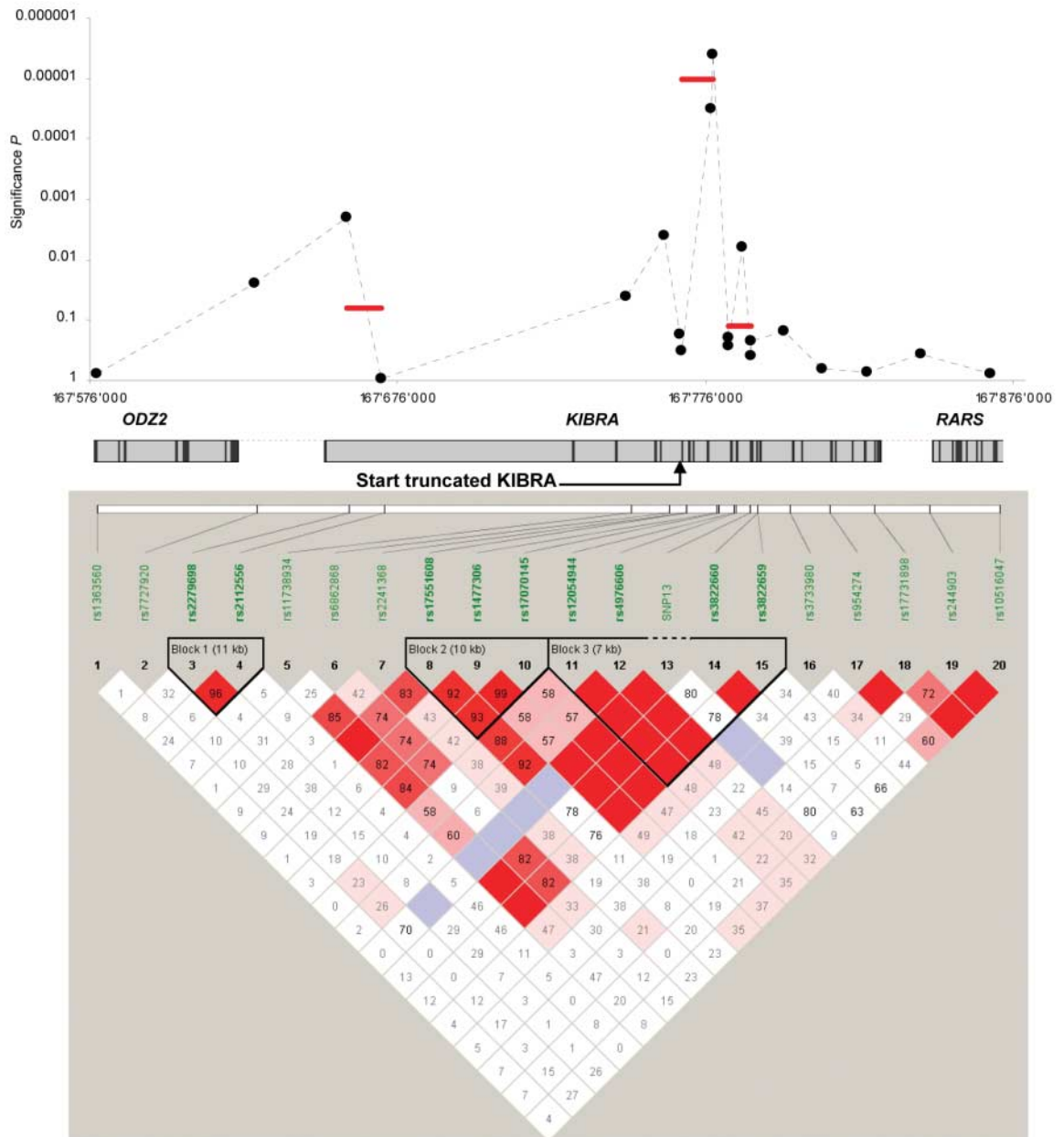
	Pictures <i>n</i> recalled after 10 min	Attention (correct characters)	Digit span (correct series)
CC	191 4.5 \pm 0.1*	157 \pm 2	8.9 \pm 0.2
CT/TT	233 5.0 \pm 0.1*	158 \pm 2	8.8 \pm 0.2

**P* = 0.006

States. The *KIBRA* SNP showed significant association with episodic memory with the same direction of effect: T allele carriers had significantly better memory scores than non-carriers in the Buschke's Selective Reminding Test (SRT) (14) (Table 2). Performance on another episodic memory task, the Rey Auditory Verbal Learning Test (AVLT) (15), was also significantly different between allele groups (Table 2). There were no allele-dependent differences in the outcome of the Wisconsin Card Sorting Test or on the Paced Auditory Serial Attention Task, suggesting that rs17070145 was not associated with executive functions, attention, or working memory in this population. As expected, the U.S. cohort displayed substantial allelic divergence. All but 18 participants reported

that they were of non-Hispanic European ancestry; the 18 exceptions were of Hispanic (9), African-American (4), Native-American (3), and Asian (2) ancestry. Exclusion of these 18 individuals had no effect on the allelic association with memory performance in this sample. Genomic control analysis showed that the exclusion of these 18 participants reduced the inflation factor λ from 1.6 to 1.2, which still indicates the presence of notable genetic heterogeneity. However, inclusion of an individual's genetic background value as a covariate had no influence on the association of the *KIBRA* genotype with episodic memory performance in this sample (10). *CLSTN2* SNP rs6439886 failed to show significant association with episodic memory in this older population

Fig. 1. Significance of SNPs and haplotypes in Swiss cohort 1. Individual genotyping in Swiss cohort 1 confirmed pooling-based screening results and implicated the *KIBRA* locus in memory performance. Black dots indicate the significance level of SNPs. Continuous red horizontal lines represent the significance level of specific haplotypes. Chromosome position in base pairs from the p terminus of chromosome 5 for each SNP is given on the x axis. Intronic regions of the *ODZ2*, *KIBRA*, and *RARS* genes are given in shaded gray, and short vertical lines represent the exons. The lower panel visualizes the haplotype structure of the examined region as assessed by Haploview 3.2 (29). Red squares represent regions of high degree of LD (*D'* > 0.8). Blue squares represent regions of high LD but for likelihood of odds (LOD) ratio.



(Table 2). The lack of significance in the U.S. population for that particular SNP may be related to differences in ethnicity or to differences in mean age between the populations.

KIBRA SNP rs17070145 was further evaluated in a third population of 424 young adults (median age, 21 years; range, 18 to 28 years) from Switzerland (Swiss cohort 2), who performed a visual episodic memory task (10). Structured association and genomic control analysis with 122 SNPs revealed a genetically homogeneous cohort ($\lambda = 1.008$). T allele carriers also performed significantly better than did noncarriers in this population (Table 3). There were no allele-dependent differences in the outcomes of the control tasks (i.e., d2 cancellation test and the digit-span task), suggesting that rs17070145 was also not associated with attention, concentration, or working memory in this population. Taken together, we found evidence for association of the T allele of rs17070145 with better episodic memory performance in three independent study populations, whereas no significant associations were found with performance in cognitive control tasks of attention, concentration, and working memory (table S2). The allelic distribution of rs17070145 differs significantly between eth-

nic groups according to the National Center for Biotechnology Information database of genetic variation (dbSNP). In populations of European ancestry, the T allele is the minor one with a frequency of ~25%, as also shown in this study. In contrast, in Asian populations the T allele is most frequent (75%) and in African-American populations, the T and C alleles are almost equally frequent (54% and 46%, respectively). Therefore, it would be interesting for subsequent studies to assess *KIBRA*'s relation to memory in populations of non-European ancestry.

Fine-mapping the genomic region harboring *KIBRA* and the flanking genes *RARS* and *ODZ2* with 19 additional SNPs in Swiss cohort 1 (Fig. 1) was performed to ensure that the observed association of *KIBRA* SNP rs17070145 with episodic memory was not due to linkage disequilibrium (LD) with genetic variations in nearby genes. Three haplotype blocks were observed within *KIBRA* with this set of SNPs (Fig. 1). SNP rs17070145 and the corresponding haplotype block (block 2) yielded the highest significance levels. We concluded that the observed association is unrelated to LD with adjacent genes (Fig. 1). Further fine-mapping of 32 kilobases encompassing *KIBRA* haplotype block 2 with 58

additional SNPs refined the haplotype structure of this region and revealed the existence of additional SNPs with significance levels comparable to those of rs17070145 (fig. S1). This can be fully attributed to the tight LD between these SNPs. As part of our quality control, the most significant *KIBRA* SNPs were genotyped twice in two independent laboratories with different singleplex genotyping technologies (Pyrosequencing and Amplifluor). Genotyping scoring was done manually and in a blind manner, and the level of congruence was >99%. Because missing genotype calls may inflate test statistics (16), we investigated the influence of calling failures on the association results of Swiss cohort 1. In 205 individually genotyped SNPs, the average calling failure rate was 3.9% for SNP assays and 2.4% for study subjects. Neither value correlated significantly with memory performance ($P > 0.5$). Inclusion of calling failures as covariate had no influence on the association of *KIBRA* SNPs with memory performance.

We determined expression levels of *KIBRA* in memory-related human brain regions with reverse transcription polymerase chain reaction (RT-PCR) amplicons designed to detect both *KIBRA* full-length transcript and its truncated form *KIAA0869*, which lacks the first 223 amino acids (17). We also studied the protein expression of truncated *KIBRA* with immunocytochemistry and Western blotting in fresh frozen brain tissue (temporal lobe) from 14 individuals (fig. S5). RNA expression levels of full-length *KIBRA* in the human brain were almost undetectable. In contrast, expression levels of truncated *KIBRA* were high in all memory-related structures in the human brain, including the hippocampus and the temporal lobe (Fig. 2). Furthermore, in situ hybridization studies in mice showed that *KIBRA* expression is highest in the dentate gyrus and the CA1 region of the hippocampal formation (Fig. 2), two key regions for memory.

Functional magnetic resonance imaging (fMRI) was done to study the relation of the *KIBRA* genotype to human memory-related neuronal activity. Thirty participants from Swiss population 1 (15 carriers of the rs17070145 T allele versus 15 noncarriers) underwent fMRI. The allelic groups were matched for sex (5 males and 10 females in each group), education ($P = 0.7$), age ($P = 0.8$), genetic heterogeneity ($P = 0.5$), and for two polymorphisms previously shown to be related to memory performance—that is, the His⁴⁵²→Tyr⁴⁵² SNP of the *HTR2A* gene ($P = 0.4$) and the Val⁶⁶→Met⁶⁶ SNP of the *BDNF* gene ($P = 0.5$). Furthermore, the groups were matched for 5-min delayed recall performance ($P = 1$) to avoid measuring genotype-unrelated performance effects on brain activations and to

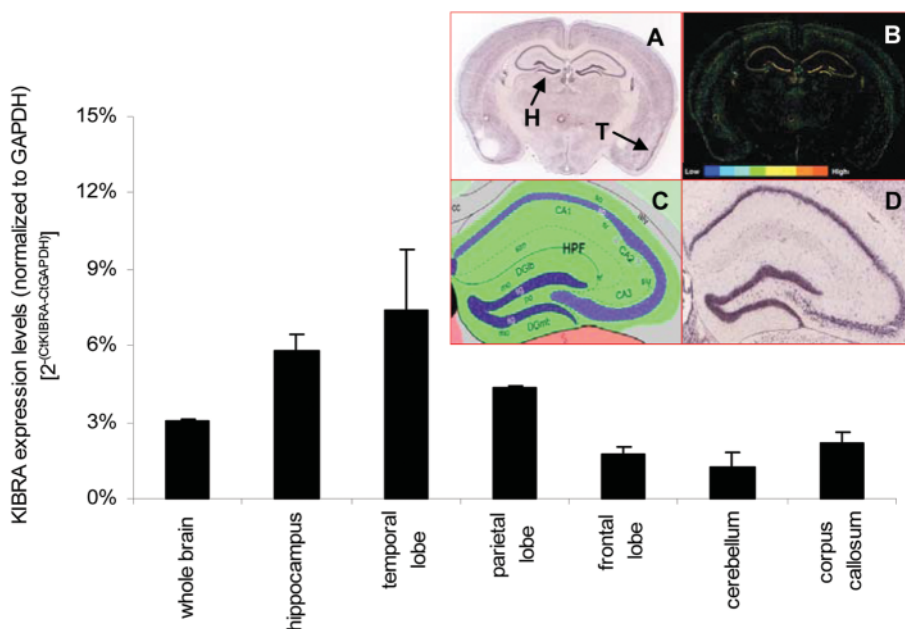


Fig. 2. *KIBRA* expression in memory-related structures in human and murine brain. Expression levels of truncated *KIBRA* in humans were measured by quantitative RT-PCR with data normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression levels. Error bars indicate SD. The inset shows the expression of *KIBRA* in the hippocampus and the temporal lobe of the murine brain as detected by in situ hybridization (30). Dark purple staining regions in (A) and (D) indicate areas of high *KIBRA* expression. (B) A gene expression filter with yellow and red color indicates areas of high gene expression. (C) An illustration of the relative atlas region. H, hippocampus; T, temporal cortex. Panels in the inset show sections scanned at high resolution (10 \times magnification, 0.95 μ m/pixel). Panel (A) shows a coronal section of the murine brain at coronal level 72, and panel (D) is a 5 \times magnification of (A) showing the hippocampal formation. Data provided under license by the Allen Institute for Brain Science.

instead capture genotype-dependent differences in brain-activation patterns. We expected that noncarriers of the T allele would need more activation in memory-related brain regions to reach the same level of memory performance (18). *KIBRA* has been associated with human episodic memory, which depends on the function of the hippocampus (13, 19, 20), leading us to also hypothesize that *KIBRA* genotypes might affect episodic memory-related information processing in the human hippocampus.

Because the hippocampus is especially activated by associative episodic memory tasks (21, 22), we tested the impact of the *KIBRA* genotype on hippocampal activations in a face-profession association task. During memory retrieval, noncarriers of the T allele showed significantly increased brain activations compared with those of T allele carriers in the medial temporal lobe [local maximum in the right hippocampus at coordinate position (26, -12, -14), $t = 4.76$, $P < 0.001$, coordinates according to the Montreal Neurological Institute] (Fig. 3). Noncarriers of the T allele also showed increased activations in the frontal cortex [local maxima in the right medial frontal gyrus (Brodmann area 8/9) at coordinate position (30, 42, 42), $t = 4.24$, $P < 0.001$ and in the left medial frontal gyrus (Brodmann area 6) at (-24, 10, 56), $t = 4.38$, $P < 0.001$] and in the parietal cortex [local maximum in the right inferior parietal lobule (Brodmann area 40) at coordinate position (50, -24, 30), $t = 3.97$, $P < 0.001$]. The brain regions observed in these studies belong to a network important for episodic memory retrieval (23), which was also activated during

our memory retrieval task (fig. S3), including the medial temporal lobe [local maximum in the right hippocampus at coordinate position (32, -8, -24), $t = 4.27$, $P < 0.001$]. There was no additional increased brain activation in noncarriers of the T allele in this episodic memory task. Furthermore, in a working memory task noncarriers of the T allele failed to show any increased retrieval-related brain activation compared with that of T allele carriers, indicating that the activations in non-carriers were specific to episodic memory retrieval.

As expected based on the matching for 5-min delayed recall performance, there were also no allele-dependent differences in retrieval performance of the fMRI task ($P = 0.5$). Our findings therefore suggest that noncarriers of the T allele need more activation in these memory retrieval-related brain regions to reach the same level of retrieval performance as T allele carriers. There was no significantly increased task-related cortical activation in the T allele group as compared to noncarriers of the T allele. No allele-dependent differences in brain activations during encoding were found, suggesting that the genotype did not affect episodic memory at this early stage of memory formation. Consistent with this observation, there were no allele-dependent differences in tasks of early memory formation in the Swiss cohort 1 (i.e., immediate recall, Table 1) or in the U.S. cohort (i.e., AVLT learning trial one, $P > 0.05$). Automated voxel-based algorithms [Statistical Parametric Mapping Software package (SPM2)] (24) and manual volume measurements failed to reveal significant allele-dependent differences in volumes of the hippocampus or the parahippocampal gyrus, or in white and gray matter volumes, suggesting that functional imaging results were not biased by morphological differences. Furthermore, there were no significant correlations between memory measures and any of the brain volumes.

Taken together, we present evidence from independent experiments suggesting a role for *KIBRA* in human memory. *KIBRA* was recently identified in a yeast two-hybrid screen as the binding partner for the human isoform of dendrin, a putative modulator of synaptic plasticity (17). A truncated form, which was expressed in the hippocampus, lacks the first 223 amino acids and contains a C2-like domain, a glutamic acid-rich stretch, and a protein kinase C (PKC) ζ -interacting domain (25). PKC ζ is involved in memory formation and in the consolidation of long-term potentiation (26, 27). The C2-like domain of *KIBRA* is similar to the C2 domain of synaptotagmin, which is believed to function as the main Ca²⁺ sensor in synaptic vesicle exocytosis (17, 28). The memory-associated *KIBRA* haplotype block and SNPs

we describe map within the truncated *KIBRA*, which contains both the C2-like and the PKC ζ -interacting domains.

References and Notes

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Supporting Online Material

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Materials and Methods

Figs. S1 to S5

Table S1

References

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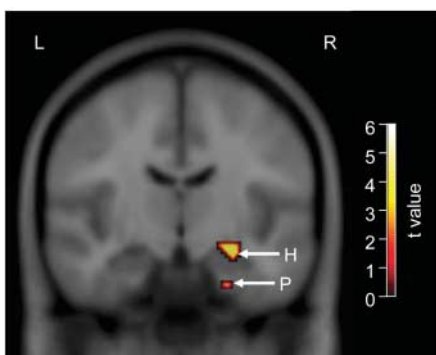


Fig. 3. Significant *KIBRA* allele-dependent differences in hippocampal activation as measured with fMRI. Activations are significantly increased in the hippocampus in noncarriers ($n = 15$) of the T allele of SNP rs17070145 than in the hippocampus of T allele carriers ($n = 15$). Activations from all 30 individuals were overlaid on a coronal section of a T1-weighted magnetic resonance image of SPM2 and displayed in color-coded t values. Threshold: $P < 0.001$. H, hippocampus; P, parahippocampal gyrus; L, left side of the brain; R, right side of the brain.