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ORIGINAL ARTICLE

# No association between *CTNNBL1* and episodic memory performance

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Polymorphisms in the gene encoding catenin-β-like 1 (*CTNNBL1*) were recently reported to be associated with verbal episodic memory performance—in particular, delayed verbal free recall assessed between 5 and 30 min after encoding—in a genome-wide association study on healthy young adults. To further examine the genetic effects of *CTNNBL1*, we tested for association between 455 single-nucleotide polymorphisms (SNPs) in or near *CTNNBL1* and 14 measures of episodic memory performance from three different tasks in 1743 individuals. Probands were part of a population-based study of mentally healthy adult men and women, who were between 20 and 70 years old and were recruited as participants for the Berlin Aging Study II. Associations were assessed using linear regression analysis. Despite having sufficient power to detect the previously reported effect sizes, we found no evidence for statistically significant associations between the tested *CTNNBL1* SNPs and any of the 14 measures of episodic memory. The previously reported effects of genetic polymorphisms in *CTNNBL1* on episodic memory performance do not generalize to the broad range of tasks assessed in our cohort. If not altogether spurious, the effects may be limited to a very narrow phenotypic domain (that is, verbal delayed free recall between 5 and 30 min). More studies are needed to further clarify the role of *CTNNBL1* in human memory.

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## INTRODUCTION

Papassotiropoulos *et al.*[1] recently reported that variants in the gene *CTNNBL1* (encoding catenin-β-like 1, located on chromosome 20 q11.23-q12) are associated with verbal episodic memory performance. The authors reached this conclusion based on the data from a genome-wide association study on 1073 healthy young adults from Switzerland; they found that the minor allele of single-nucleotide polymorphism (SNP) rs16986890 was significantly ($P = 7E-08$) associated with better verbal episodic memory performance. This result was corroborated in a second and independent cohort of young adults from Serbia ($n = 524$, $P = 0.003$), although a different, nontypical experimental paradigm was used to assess episodic memory performance in that data set. In addition to these genetic association results, the authors also provided evidence from both gene expression data and functional magnetic resonance imaging experiments to support the notion that rs16986890 in *CTNNBL1* may account for genotype-dependent differences in memory-related brain functions.

Although the results reported by Papassotiropoulos *et al.*[1] were consistent for young and highly educated adults, no information was provided as to whether or not they can be generalized to adults with a wider range of ages and more diverse educational backgrounds. Moreover, their findings were based mainly on relatively simple verbal recall tasks. Because the concept of episodic memory has a number of other aspects beyond verbal recall, a more comprehensive assessment of this general phenotype is needed for a better understanding of the potential role(s) of genetic determinants in episodic memory.

The present study represents the first independent attempt to replicate the role of rs16986890 in human episodic memory performance following the initial report. In addition, we substantially extend the analyses of Papassotiropoulos *et al.*[1] by investigating the potential effects of 454 other SNPs in or near *CTNNBL1*, and by testing a broad range of verbal and nonverbal episodic memory tasks in both young and old adults. Assessments were performed in a large and independent genome-wide association study sample from Germany ($n = 1743$), collected as part of the Berlin Aging Study II. Despite having sufficient power to replicate findings of the original report, we found no evidence for a genetic link between *CTNNBL1* and episodic memory performance in our cohort.

## MATERIALS AND METHODS

### Participants

Our sample was recruited as participants in the Berlin Aging Study II,[2] a multidisciplinary project aiming to identify and characterize genetic, psychological, medical and socioeconomic factors relevant to human aging in residents of Berlin, Germany. The sample currently comprises 1946 genetically unrelated, mentally healthy individuals, all of whom were of self-reported Caucasian ancestry. The study was approved by the local Institutional Review Board, and all participants gave signed informed consent before participation. Among the 1743 probands available for analysis in our study, 425 were young adults aged between 20–30 years and the remaining 1318 were old adults aged between 60–70 years.

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## Assessment of episodic memory performance

To assess the role of *CTNNBL1* in human memory, we used 14 quantitative measures of episodic memory derived from: (a) the forward and backward serial recall paradigms;[3,4] (b) associative memory tasks that assessed item memory as well as item–pair recognition;[5] and (c) an image recognition test at retention intervals of 2.5 and 1 h.[6] A detailed description of the tasks and traits analyzed can be found in Supplementary Table 1. For a description on how multicollinearity among traits is dealt with, see description of the statistical analysis procedures below.

*Forward and backward serial recall task*. In this task, participants were presented with six different lists of 12 words each. After the presentation of the last item in each list, participants were asked to recall each word at its correct position. Word lists 1–3 were recalled in forward order (from the first word to the last word of the list), whereas word lists 4–6 were recalled in backward order. Recall was self-paced. For each list, responses were scored using a strict serial recall criterion: an accurate response required that both the word and its serial position were correct.

*Item and pair associative episodic memory task*. The item and pair associative episodic memory task (henceforth labeled as the 'item–pair' task) had four conditions that were tested sequentially in one session. During an initial study phase, participants were visually presented with pairs of unrelated words and were instructed to study each pair under two conditions: either as two single words (item instruction) or as a pair of words (pair instruction).

The study phase of each condition contained 30 pairs of semantically unrelated words. In the test phrase, subjects in one condition (item recognition) were asked whether they had seen the presented word during the study phase. Half of the words were old (target items), and the other half were new (distractor items). In the second condition (associative recognition), subjects had to decide whether a presented pair of words had been presented during the study phase. Half of the presented word pairs were old (target pairs), and the others were formed by recombining words in the previously studied lists (rearranged distractor pairs). Taken together, by crossing over the two instruction conditions with the two test conditions, the task resulted in four conditions that assessed item memory (item–item and pair–item tests) and associative memory (pair–pair and item–pair tests). Recognition memory performance was measured as hits minus false alarms to minimize the effects of potential individual differences in response bias.

*Image recognition memory with retention*. Performance in the image recognition memory task was assessed at two retention intervals: 2.5 h and 1 week. At the beginning of the first session, participants were presented with 48 complex, colored images of scenes of neutral emotional valence; all were derived from the International Affective Picture System.[7]

The images were encoded incidentally: during the study phase, participants were required to determine whether the scene was 'indoor' or 'outdoor'—there were 24 scenes in each category—without explicit requirement of memorization. During retrieval, participants viewed each image for 3 sec and were asked to determine whether each scene had been presented ('old') or not ('new') during encoding. In each retrieval test, 24 unique old scenes and 24 unique new scenes (lures) were presented. Taking response bias into account, memory performance was measured as hits minus false alarms.

## Genotyping, SNP imputation and quality control procedures

DNA of each participant was extracted from whole blood using standard procedures, and it was then subject to microarray-based SNP genotyping using the Affymetrix 'Genome-Wide Human SNP Array 6.0'. Before imputation, SNPs violating Hardy–Weinberg equilibrium at $P \leqslant 1E-06$ and those with a call rate $< 98\%$—two commonly used quality control filters—were excluded. This resulted in 829 344 autosomal SNPs in 1946 participants.

Among these individuals, 214 were excluded from subsequent analysis. Each of them had at least one of the following conditions: (a) missing information on age or years of education; (b) $< 95\%$ call rate; (c) evidence for sample duplication, relatedness or contamination; (d) inconsistency between recorded and genotypic sex; (e) excessive heterozygosity; and (f) population outlier, which was determined by the EIGENSOFT program;[8] specifically, because all participants were of self-reported Caucasian descents, we excluded ethnic outliers using the Eigenstrat function in EIGENSOFT with iterative outlier removal. After the above filtering steps, principal components (PCs) were computed again for the 1743 remaining samples. On the basis of the examination of the scree plot, the first four PCs were retained and used as covariates for the subsequent association analysis, to adjust for potential residual population stratification.

Genome-wide imputation of unobserved genotypes was carried out on the 'cleaned' data set using the IMPUTE v2.3.2 software,[9,10] on the basis of the precompiled '1000 Genomes Phase I Integrated Variant Set' reference panels from the IMPUTE website (March 2012 release). As suggested by Southam *et al*,[11] we also applied post-imputation quality control filtering, including only SNPs with IMPUTE- information thresholds $\geqslant 0.8$ and minor allele frequencies at or above 5%. After this post-imputation filtering, a total of 455 high-quality SNPs, 71 genotyped and 384 imputed, within a $\pm 50$ kb window surrounding the *CTNNBL1* locus (that is, between start bp 36 272 434 and end bp 36 550 520; hg19 human reference genome assembly) were retained for subsequent statistical analyses.

## Association analyses

The phenotypes we used for evaluating participants' episodic memory performance are functionally related and statistically correlated. The 14 phenotypes are correlated with an average correlation coefficient (r) of 0.46. One strategy to analyze these data is to test each SNP against each of the 14 phenotypes individually. Although this approach is straightforward, it is limited by not incorporating potentially useful information from the structure of multiple (and partially correlated) phenotypes. To address this issue, we also used a second approach that can test several correlated phenotypes simultaneously. As suggested by a recent review,[12] we first applied principal component analysis (PCA) to condense information in the phenotypes by extracting a small number of orthogonal variables (that is, the PCs) that were weighted linear combinations of the original phenotypes. The extracted variables, which were the first three PCs from PCA, were then used for association analyses in place of the original phenotypes. As expected, the three components were correlated to the three different episodic memory tasks, and altogether they could explain 80% of the phenotypic variance.

Association analyses were carried out using the episodic memory measures (that is, each trait individually and the PCA variables) as quantitative traits in an additive linear model, adjusted for age, gender, years of education, as well as the four PCs to account for potential population stratification. All analyses were performed separately in the 'old' and 'young' strata, to avoid stratification problems. Association tests were performed using SNPTEST v.1.3,[13] which can account for the uncertainty of imputed genotype calls via missing data likelihood tests.

## Power calculations

The power of our study was assessed in the young and old subgroups separately and was based on the reported effect sizes from the original study.[1] Monte Carlo simulations were performed with 1000 runs for empirical power calculations. For the one-trait-at-a-time approach, we used the SimpleM software[14,15] to account for the correlations among the 14 test items and the correlations among SNPs due to linkage disequilibrium.[16] The results of this analysis, that is, the effective number of independent tests, were then used for Bonferroni-correction to account for multiple testing.[16] The estimated effective number of independent tests was 11 and 60 across the 14 phenotypes (or traits) and 455 SNPs, respectively. Hence, the experiment-wide corrected alpha level was set to $4.55E-03$ (that is, 0.05/11), for testing the association between at least one of the traits and the previously reported significant SNP rs16986890; and it was $7.58E-05$ (that is, $0.05/(11 \times 60)$) for testing the association between at least one of the traits and any SNP in the *CTNNBL1* gene region.

Our power to detect an association between at least one of the traits and rs16986890 at the originally reported effect size was between 93% and 100% for the 'young' stratum and 100% for the 'old' stratum (see Table 1). When we extended our search to the whole *CTNNBL1* gene region, the power to detect association between at least one of the traits and any of the 455 SNPs in the *CTNNBL1* gene region was between 61% and 97% for the 'young' stratum and 100% for the 'old' stratum (see Table 2). With the PCA approach, our power to detect association between at least one of the three PCs (that is, the extracted variables from PCA of the 14 traits) and rs16986890 was between 90% and 100% for the 'young' stratum and 100% for the 'old' stratum; and it was between 56% and 96% for the 'young' stratum and 100% for the 'old' stratum when testing possible associations between at least one of the PCs and any SNP in the *CTNNBL1* gene region.

**Table 1.** Association results for rs16986890 with episodic memory performance measures in the BASE-II cohort

| *Trait* | *Mean (AA)* | *Mean (AG)* | *Mean (GG)* | P-*raw* | P-*adjusted* | *Beta* | *s.e.* |
|---|---|---|---|---|---|---|---|
| SRFOBK (young) | 0.6063 | 0.5927 | 0.7691 | 0.4984 | 1 | −0.0983 | 0.1452 |
| SRFOBK (old) | 0.4643 | 0.4519 | 0.4483 | 0.0605 | 0.6655 | −0.1592 | 0.0848 |
| SRFO (young) | 0.6033 | 0.5874 | 0.7896 | 0.4897 | 1 | −0.1002 | 0.1450 |
| SRFO (old) | 0.4654 | 0.4536 | 0.4618 | 0.0761 | 0.8371 | −0.1500 | 0.08453 |
| SRFO_P1 (young) | 0.8323 | 0.8557 | 0.9200 | 0.5249 | 1 | 0.0937 | 0.1474 |
| SRFO_P1 (old) | 0.6020 | 0.5928 | 0.5400 | 0.7670 | 1 | −0.0253 | 0.08557 |
| SRFO_P2 (young) | 0.5691 | 0.5250 | 1.0000 | 0.3241 | 1 | −0.1422 | 0.1441 |
| SRFO_P2 (old) | 0.2144 | 0.1859 | 0.1250 | 0.1437 | 1 | −0.1231 | 0.08424 |
| SRFO_P3 (young) | 0.5622 | 0.5311 | 1.0000 | 0.7540 | 1 | −0.0460 | 0.1467 |
| SRFO_P3 (old) | 0.2749 | 0.2420 | 0.3300 | 0.0756 | 0.8316 | −0.1513 | 0.08513 |
| SRBK (young) | 0.5958 | 0.5861 | 0.7140 | 0.5893 | 1 | −0.0789 | 0.1460 |
| SRBK (old) | 0.4676 | 0.4566 | 0.4417 | 0.1229 | 1 | −0.1322 | 0.08572 |
| SRBK_P1 (young) | 0.7007 | 0.6848 | 1.0000 | 0.8337 | 1 | −0.0307 | 0.1463 |
| SRBK_P1 (old) | 0.3773 | 0.3367 | 0.2500 | 0.0713 | 0.7843 | −0.1566 | 0.08686 |
| SRBK_P2 (young) | 0.6483 | 0.6111 | 1.0000 | 0.4527 | 1 | −0.1101 | 0.1466 |
| SRBK_P2 (old) | 0.2649 | 0.2440 | 0.2500 | 0.4167 | 1 | −0.0693 | 0.08544 |
| SRBK_P3 (young) | 0.7922 | 0.7783 | 0.9200 | 0.7465 | 1 | −0.0477 | 0.1475 |
| SRBK_P3 (old) | 0.6433 | 0.6136 | 0.6650 | 0.0559 | 0.6149 | −0.1625 | 0.08471 |
| Delay_2.5 h (young) | 0.6928 | 0.6987 | 0.9200 | 0.9458 | 1 | 0.0099 | 0.1453 |
| Delay_2.5 h (old) | 0.6275 | 0.6393 | 0.5800 | 0.5163 | 1 | 0.0556 | 0.0886 |
| Delay_1 week (young) | 0.4563 | 0.4951 | 0.2400 | 0.4998 | 1 | 0.0982 | 0.1455 |
| Delay_1 week (old) | 0.2905 | 0.2544 | 0.2100 | 0.0061 | 0.0671 | −0.2317 | 0.08444 |
| Item–item (young) | 0.6142 | 0.6385 | 1.0000 | 0.4917 | 1 | 0.0974 | 0.1416 |
| Item–item (old) | 0.5175 | 0.5234 | 0.6350 | 0.3906 | 1 | 0.0723 | 0.08412 |
| Item–pair (young) | 0.3555 | 0.2947 | 0.8700 | 0.2817 | 1 | −0.1576 | 0.1463 |
| Item–pair (old) | 0.1709 | 0.2070 | 0.1800 | 0.1322 | 1 | 0.1288 | 0.08585 |
| Pair–pair (young) | 0.6349 | 0.5411 | 0.9300 | 0.0775 | 0.8525 | −0.2570 | 0.1453 |
| Pair–pair (old) | 0.3998 | 0.3822 | 0.4850 | 0.6579 | 1 | −0.0369 | 0.08321 |
| PC1 (young) | −3.0145 | −2.6460 | −7.3921 | 0.4336 | 1 | 0.3117 | 0.3980 |
| PC1 (old) | 0.9629 | 1.2765 | 1.2932 | 0.0867 | 0.2601 | 0.2818 | 0.1644 |
| PC2 (young) | 0.03176 | 0.2000 | 0.1757 | 0.7019 | 1 | 0.0584 | 0.1526 |
| PC2 (old) | −0.02567 | 0.06954 | −0.07147 | 0.3976 | 1 | 0.0789 | 0.09339 |
| PC3 (young) | −0.2131 | −0.4852 | 1.4498 | 0.3237 | 0.9711 | −0.1601 | 0.1621 |
| PC3 (old) | 0.05758 | 0.26661 | 0.8522 | 0.0213 | 0.0639 | 0.2054 | 0.08938 |

Abbreviations: BASE-II, Berlin Aging Study II; P1, P2 and P3 are the first, second and third portion of the recall paradigms; *P*-adjusted, *P*-value after adjusting for multiple comparisons (see methods); *P*-raw, nominal *P*-value of association statistic; SRFOBK, combined accuracy of forward and backward serial recall tests; SRFO, accuracy of forward serial recall test; SRBK, accuracy of backward serial recall test. For more detailed descriptions of the tasks and traits, please see Supplementary Methods and Supplementary Table 1. For more details on the administration of these tests, see studies by Lewandowsky *et al*.,[3] Li *et al*.[17] and Papenberg *et al*.[6] Phenotypic means of genotypes AA (1128–1173 old/373–377 young), AG (137–142 old/45–47 young) and GG (4 old/1 young) are shown in columns 2, 3 and 4, respectively.

## RESULTS

After quality control and adjusting for potential population stratification, there was only minimal evidence of *P*-value inflation: $\lambda$ ranged from 1.00–1.04 across the 14 traits tested in the 'young' and 'old' strata. The minor allele frequency of rs16986890 was 0.056 and 0.058 for the 'young' and the 'old' in our German sample, respectively, which are consistent with data (that is, 0.058) reported by the 1000 Genomes Project for European (CEU) samples.[18]

As seen in Table 1, there was no evidence that SNP rs16986890, which elicited the strongest signal in the original report,[1] was associated with any of the 14 memory measures after correcting for multiple comparisons. Adjusted results approached experiment-wide significance (corresponding to a nominal *P*-value of 4.55E − 03, see Materials and Methods) for two memory measures ('delay_1_week' ($P = 0.0671$) and 'PC3' (0.0639)) in the 'old' stratum. However, the directions of these effects were opposite to what was reported in the original study, suggesting that worse, instead of better, memory performance was related to the minor allele of rs16986890. Moreover, as seen in the local chromosome region views in Figure 1 and the Supplementary Figures, this 'signal' was indistinguishable from noise. Finally, there was no evidence that any of the other 454 SNPs was significantly associated with the memory measures (all adjusted *P*-values $> 0.05$ (corresponding to a nominal *P*-value of 7.58E − 05, see Materials and Methods)). Results obtained using the PCA approach were very similar to those obtained with the one-trait-at-a-time analyses. Reanalysis of all comparisons without adjusting for years of education—which might themselves have a weak genetic component[19] did not change the results appreciably (data not shown). Summaries of the full results can be found in Table 1, Table 2, Figure 1 and the Supplementary Figures.

## DISCUSSION

In this study, we comprehensively investigated the potential effects of common genetic variants in or near *CTNNBL1* on a broad range of verbal and nonverbal episodic memory tasks for both young and old adults. Assessments were performed in over 1700 individuals recruited as part of the Berlin Aging Study II. In contrast to the recently reported findings by Papassotiropoulos *et al*.,[1] our independent data provide no support for the notion that SNPs in the *CTNNBL1* gene region, including rs16986890, exert any significant effects on episodic memory performance. This conclusion holds true regardless of whether we considered the various tested cognitive traits individually or in combined analyses. Likewise, we saw no evidence of association with respect to age. To the best of our knowledge, this is the first independent replication attempt since the publication of the original report. On the basis of our data, it remains highly doubtful that genetic

**Table 2.** Association results for the most significant of 455 SNPs in *CTNNBL1* with episodic memory performance measures in the BASE-II cohort

| *Trait* | *rs number* | *Position* | *A1* | *A2* | *Maf* | P-*raw* | P-*adjusted* | *Beta* | *s.e.* |
|---|---|---|---|---|---|---|---|---|---|
| SRFOBK (young) | rs913966 | 36309922 | G | C | 0.06179 | 0.05215 | 1 | −0.2877 | 0.1476 |
| SRFOBK (old) | rs6095709 | 36305770 | G | A | 0.07161 | 0.00556 | 1 | −0.2320 | 0.08374 |
| SRFO (young) | rs6063608 | 36430407 | G | A | 0.2170 | 0.07103 | 1 | −0.1430 | 0.07904 |
| SRFO (old) | rs6095709 | 36305770 | G | A | 0.07189 | 0.00992 | 1 | −0.2148 | 0.08332 |
| SRFO_P1 (young) | rs1123291 | 36529854 | G | A | 0.06437 | 0.02447 | 1 | 0.3400 | 0.1504 |
| SRFO_P1 (old) | rs198490 | 36273719 | C | T | 0.4420 | 0.07607 | 1 | −0.07010 | 0.03948 |
| SRFO_P2 (young) | rs6091461 | 36530906 | A | G | 0.2691 | 0.04901 | 1 | −0.1571 | 0.07968 |
| SRFO_P2 (old) | rs62201719 | 36517381 | A | G | 0.4643 | 0.0220 | 1 | 0.08988 | 0.03922 |
| SRFO_P3 (young) | rs913966 | 36309922 | G | C | 0.06179 | 0.04872 | 1 | −0.2934 | 0.1483 |
| SRFO_P3 (old) | rs6095709 | 36305770 | G | A | 0.07161 | 0.01647 | 1 | −0.2021 | 0.08441 |
| SRBK (young) | rs913966 | 36309922 | G | C | 0.06179 | 0.03835 | 1 | −0.3092 | 0.1486 |
| SRBK (old) | chr20:36488508:D | 36488508 | TCTCA | T | 0.2581 | 0.01418 | 1 | 0.1111 | 0.04525 |
| SRBK_P1 (young) | rs11698255 | 36531400 | G | A | 0.07086 | 0.01179 | 1 | 0.3643 | 0.1438 |
| SRBK_P1 (old) | rs2144768 | 36501221 | G | A | 0.2809 | 0.00200 | 1 | 0.1437 | 0.04640 |
| SRBK_P2 (young) | rs913966 | 36309922 | G | C | 0.06179 | 0.11507 | 1 | −0.2382 | 0.1511 |
| SRBK_P2 (old) | rs73290822 | 36325944 | C | T | 0.05222 | 0.05189 | 1 | 0.1739 | 0.08966 |
| SRBK_P3 (young) | rs913966 | 36309922 | G | C | 0.06179 | 0.02859 | 1 | −0.31934 | 0.1453 |
| SRBK_P3 (old) | rs75642402 | 36423858 | T | C | 0.06549 | 0.00502 | 1 | −0.24575 | 0.0880 |
| Delay_2.5 h (young) | rs1535184 | 36525522 | T | C | 0.2024 | 0.02358 | 1 | −0.20769 | 0.09165 |
| Delay_2.5 h (old) | rs1535183 | 36525517 | A | G | 0.2951 | 0.00853 | 1 | 0.1201 | 0.04565 |
| Delay_1 week (young) | rs73290839 | 36356451 | A | G | 0.06818 | 0.03357 | 1 | 0.3082 | 0.1444 |
| Delay_1 week (old) | rs59989754 | 36400997 | G | A | 0.05085 | 0.00097 | 0.64 | −0.3040 | 0.09191 |
| Item–item (young) | rs73290827 | 36335697 | T | G | 0.06673 | 0.00169 | 1 | −0.4443 | 0.1381 |
| Item–item (old) | rs112689619 | 36278748 | T | C | 0.05559 | 0.03147 | 1 | 0.1907 | 0.08881 |
| Item–pair (young) | rs75642402 | 36423858 | T | C | 0.06789 | 0.04421 | 1 | −0.3036 | 0.1512 |
| Item–pair (old) | rs6126493 | 36532374 | C | T | 0.4285 | 0.00503 | 1 | −0.1174 | 0.04184 |
| Pair–pair (young) | rs62201726 | 36543271 | G | A | 0.1004 | 0.00186 | 1 | −0.3684 | 0.1187 |
| Pair–pair (old) | chr20:36283430:D | 36283430 | TG | T | 0.1947 | 0.15125 | 1 | −0.07110 | 0.04972 |
| PC1 (young) | rs913966 | 36309922 | G | C | 0.06048 | 0.044565 | 1 | 0.8207 | 0.4070 |
| PC1 (old) | rs73290822 | 36325944 | C | T | 0.05165 | 0.015473 | 1 | −0.4290 | 0.1770 |
| PC2 (young) | rs913966 | 36309922 | G | C | 0.06048 | 0.01624 | 1 | 0.3811 | 0.1578 |
| PC2 (old) | rs1535183 | 36525517 | A | G | 0.2937 | 0.009717 | 1 | 0.1296 | 0.05010 |
| PC3 (young) | rs66999026 | 36414507 | G | A | 0.06705 | 0.004033 | 0.7259 | −0.4666 | 0.1597 |
| PC (old) | rs6096781 | 36527215 | T | C | 0.05276 | 0.002951 | 0.5312 | 0.2767 | 0.09290 |

Abbreviations: A1, allele 1; A2, allele 2; BASE-II, Berlin Aging Study II; MAF, minor allele frequencies in the respective cohort (that is, 'old' or 'young'); P1, P2 and P3 are the first, second and third portion of the recall paradigms; *P*-adjusted, *P*-value after adjusting for multiple comparisons (see methods); *P*-raw, nominal *P*-value of association statistic; SRFOBK, combined accuracy of forward and backward serial recall tests; SRFO, accuracy of forward serial recall test; SRBK, accuracy of backward serial recall test. For more detailed descriptions of the tasks and traits, please see Supplementary Methods and Supplementary Table 1. For more details on the administration of these tests, see studies by Lewandowsky *et al*.,[3] Li *et al*.[17] and Papenberg *et al*.[6] Phenotypic means of genotypes AA (1128–1173 old/373–377 young), AG (137–142 old/45–47 young) and GG (4 old/1 young) are shown in columns 2, 3 and 4, respectively. Position is the SNP location on chromosome 20 based on hg19.

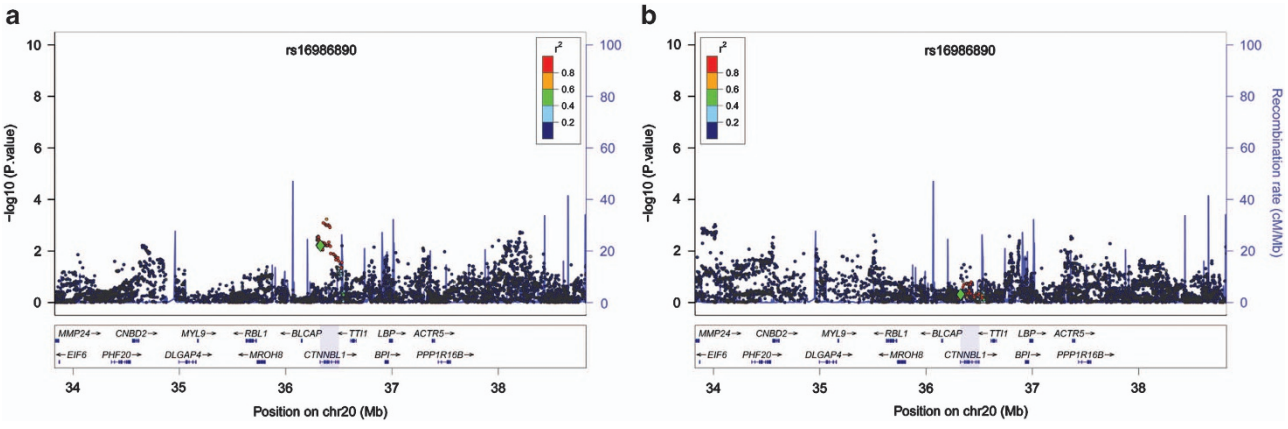


**Figure 1.** Local view of association signals in the chromosomal region around *CTNNBL1* using the delayed image recognition paradigm. (**a**) Results from association analyses of 455 SNPs in the *CTNNBL1* region and episodic memeory performance of the 'old' (between 60 and 70 years old) subgroup of BASE-II participants. (**b**) Results from association analyses of 455 SNPs in the *CTNNBL1* region and episodic memory performance of the 'young' (between 20 and 30 years old) subgroup of BASE-II participants. In both plots, SNPs are plotted by chromosomal position against association with 'delay_1_week' (− log10 P). The index SNP rs16986890 is denoted by a green diamond and the location of gene *CTNNBL1* is highlighted in gray color below the plots. SNPs are colored to reflect linkage disequilibrium with rs16986890 (using the CEU 1000 Genomes Project for European panel from HapMap Phase II). Images were generated using LocusZoom (http://csg.sph.umich.edu/locuszoom/). BASE-II, Berlin Aging Study II; SNP, single-nucleotide polymorphism.

variants in *CTNNBL1* are genuinely involved in mechanisms controlling episodic memory in humans.

The reason for the observed discrepancy between our results and those from the original paper remain elusive. Although we did not apply the same memory tests (that is, verbal delayed free recall between around 5 and 30 min) as in Papassotiropoulos *et al*.,[1] our assessments cover a wide range of related tasks making it unlikely that we have missed a strong general effect of *CTNNBL1* on episodic memory. Most of the tests applied to our participants can be considered more challenging than the free recall tests used in the original study. For instance, the serial recall tasks place more demand on associative memory than free recall, which relies mainly on item memory.[3,17] Likewise, the delayed image recognition test used here[6] involves a much longer retention interval than 30 min as applied in the original study. Therefore, we cannot rule out the possibility that a potential effect of SNP rs16986890 (or other SNPs in the *CTNNBL1* region) is limited to a very narrow phenotypic domain, that is, verbal delayed free recall between around 5 and 30 min.

In summary, our study provides considerable negative evidence against the notion that genetic variants in *CTNNBL1* are associated with episodic memory performance. More independent assessments with sufficiently large sample sizes are needed to further clarify the potential role, if any, of this gene in human memory.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)