Expression of TCN1 in Blood is Negatively Associated with Verbal Declarative Memory Performance

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Memory is indispensable for normal cognitive functioning, and the ability to store and retrieve information is central to mental health and disease. The molecular mechanisms underlying complex memory functions are largely unknown, but multiple genome-wide association studies suggest that gene regulation may play a role in memory dysfunction. We performed a global gene expression analysis using a large and balanced case-control sample (n = 754) consisting of healthy controls and schizophrenia and bipolar disorder patients. Our aim was to discover genes that are differentially expressed in relation to memory performance. Gene expression in blood was measured using Illumina HumanHT-12 v4 Expression BeadChip and memory performance was assessed with the updated California Verbal Learning Test (CVLT-II). We found that elevated expression of the vitamin B12-related gene TCN1 (haptocorrin) was significantly associated with poorer memory performance after correcting for multiple testing (β = −1.50, p = 3.75e-08). This finding was validated by quantitative real-time PCR and followed up with additional analyses adjusting for confounding variables. We also attempted to replicate the finding in an independent case-control sample (n = 578). The relationship between TCN1 expression and memory impairment was comparable to that of important determinants of memory function such as age and sex, suggesting that TCN1 could be a clinically relevant marker of memory performance. Thus, we identify TCN1 as a novel genetic finding associated with poor memory function. This finding may have important implications for the diagnosis and treatment of vitamin B12-related conditions.

The capacity to form short and long-term memories is indispensable for the normal functioning of our cognitive abilities, and the central role played by memory impairment in the development of severe psychiatric disorders is well established1,2. For these reasons, the molecular underpinnings of memory formation and retrieval have been studied extensively, and basic molecular mechanisms underlying memory function have been identified3,4. However, most of what we know about the molecular biology of memory is confined to relatively simple memory performances involving simple sensory stimuli like tone, touch, or shock in invertebrates and non-human vertebrates1. The molecular basis of more complex forms of memory, such as human declarative memory, which involves the conscious recollection of factual information or previous experiences, remains largely unknown1.

Genome-wide association studies (GWAS) have discovered multiple genetic variants associated with memory performance6–8. An interesting aspect of these studies is the large proportion of associated loci that are located in non-coding regions of the genome (intergenic or intronic). This indicates that the genetic architecture of complex memory functions primarily involves variants that exert their effects through regulating gene expression rather than altering protein structure and function6. Studying the transcriptional profiles of declarative memory performance may thus provide useful insights into the initial links between statistical genetic associations and cellular pathways. Moreover, such insights may help to elucidate the complex molecular mechanisms underlying severe memory impairments.

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psychiatric illnesses like schizophrenia (SCH) and bipolar disorder (BD), since both disorders are strongly characterized by cognitive dysfunction in general and memory impairment in particular. In the present study, we explored the genome-wide transcriptional basis of verbal declarative memory. This kind of declarative memory involves the verbal recall of facts and events and is among the most affected cognitive domains in SCH and BD. Previous studies examining the expression profile of memory have generally been hypothesis-driven, limiting themselves to investigating the regulatory patterns of candidate genes instead of global expression patterns. Additionally, most of these studies examined the association between gene expression and general cognitive impairment rather than specifically measuring verbal declarative memory. One exception is a recent paper by Zheutlin et al., in which genome-wide peripheral expression with regard to verbal memory was assessed. However, this study was limited by a relatively small sample size (n = 190) and a lack of adjustment for critical variables, such as medication use, which is known to influence both gene expression and cognitive performance. Addressing these limitations may therefore increase the power to identify differentially expressed genes of smaller effect sizes, as well as help to distinguish the real effects of altered expression levels from those of secondary factors. The aim of our study was three-fold. First, we performed a global gene expression analysis in peripheral blood using a large case-control sample (n = 754) aiming to discover novel genes that are differentially expressed in relation to memory performance. Second, we sought to replicate any significant finding in a non-overlapping sample (n = 578) drawn from the same geographical area. Finally, we followed up any discovery with biological relevance and strong statistical significance with additional analyses, adjusting for potentially confounding variables.

**Results**

**Genome-wide screening of expression markers.** An initial genome-wide expression analysis was performed to identify associations between 23,476 genetic markers and two memory test scores. The verbal learning measure (CVLT1; Table 1) was significantly associated with one marker after adjusting for multiple testing (α = 1.06e-6): the HLA-DRA probe ILMN_2157441 (β = 6.57, p = 4.90e-7, 95% CI [4.03, 9.11]). The long delay free recall measure (CVLT2; Table 2) was only significantly associated with the TCN1 marker (β = −1.50, p = 3.75e-8, 95% CI [−2.02, −0.97]). TCN1 was also among the top ten genes associated with CVLT1 performance (β = −4.43, p = 4.42e-6, 95% CI [−6.30, −2.55]). The variance in memory performance explained by TCN1 expression was comparable in the two models (partial $R^2_{CVLT1} = 0.030$; partial $R^2_{CVLT2} = 0.043$). Furthermore, TCN1 had a negative correlation with both test scores (CVLT1: Pearson’s R = −0.220, 95% CI [−0.290, −0.148]; CVLT2: Pearson’s R = −0.247, 95% CI [−0.316, −0.176]; Fig. 1). Two different markers targeting the gene RNA18S5 were among the top ten candidates in both memory scores, but these markers did not survive correction for multiple testing (Tables 1 and 2).

**Table 1. Top Ten Associations between Gene Expression and Verbal Learning (CVLT1).** Std $\beta$: Standardized regression coefficients. *Bonferroni-adjusted p-value < 0.05.

| Probe name | Gene symbol | Gene name | Gene function | $\beta$  | 95% CI          | Std $\beta$ | p-value Nominal | p-value Bonferroni | Adjusted $R^2$ |
|------------|-------------|-----------|---------------|---------|----------------|-------------|-----------------|-------------------|----------------|
| ILMN_2157441 | HLA-DRA     | Major histocompatibility complex, class II, DR alpha | Immune system, antigen presentation | 6.57    | 4.03, 9.11     | 0.56        | 4.90e-07        | 0.023*            | 0.101           |
| ILMN_2114720 | SLP       | Secretory leukocyte peptidase inhibitor | Immune system, protection of epithelial tissues from serine proteases | −5.70   | −8.01, −3.39   | −0.49       | 1.56e-06        | 0.073             | 0.098           |
| ILMN_3239610 | RNA18S5    | RNA, 18S ribosomal 5 | Structural ribosomal component | −2.39   | −3.39, −1.40   | −0.20       | 2.88e-06        | 0.135             | 0.097           |
| ILMN_1703337 | RNA18S5    | RNA, 18S ribosomal 5 | Structural ribosomal component | −2.64   | −3.75, −1.53   | −0.23       | 3.37e-06        | 0.158             | 0.097           |
| ILMN_1712888 | HSPH1     | Heat shock protein family H member 1 | Protein folding | 7.22    | 4.18, 10.27    | 0.62        | 3.72e-06        | 0.175             | 0.096           |
| ILMN_2041046 | CKS1B     | CDC28 protein kinase regulatory subunit 1B | Cell cycle regulation | 12.41   | 7.15, 17.67    | 1.06        | 4.32e-06        | 0.203             | 0.096           |
| ILMN_1768469 | TCN1      | Transcobalamin 1; haptocorrin | Vitamin B12 transportation and cellular uptake | −4.43   | −6.30, −2.55   | −0.38       | 4.42e-06        | 0.207             | 0.096           |
| ILMN_1812433 | HP         | Haptoglobin | Binding of free plasma hemoglobin | −7.30   | −10.52, −4.07  | −0.62       | 1.06e-05        | 0.486             | 0.094           |
| ILMN_1750661 | FBXW9     | F-box and WD repeat domain containing 9 | Ligation of ubiquitin to proteins | −13.40  | −19.44, −7.35  | −1.14       | 1.57e-05        | 0.737             | 0.093           |
| ILMN_3251587 | RNA28S5   | RNA, 28S ribosomal 5 | Structural ribosomal component | −1.52   | −2.22, −0.83   | −0.13       | 1.79e-05        | 0.843             | 0.092           |

Differential expression between diagnostic groups. We found a significantly different expression across diagnostic groups for both TCN1 (F(2,2751) = 20.87, p = 1.5e-9, $R^2 = 0.050$) and HLA-DRA (F(2,2751) = 26.50, p = 7.54e-12, $R^2 = 0.063$). HLA-DRA had significantly reduced expression in the BD (mean = 12.67, sd = 0.32, p = 2.14e-9) and SCH (mean = 12.67, sd = 0.33, p = 4.62e-10) groups compared to the CTRL group (mean = 12.86, sd = 0.31), but no significant difference was found between the patient groups.
(p = 0.99; see Supplementary Figure S1), suggesting that the underlying immune-related mechanisms are partly similar in the two disorders. Expression of TCN1 was significantly elevated in SCH (mean = 7.87, sd = 0.48) compared to both BD (mean = 7.73, sd = 0.48) and CTRL (mean = 7.61, sd = 0.37). All group differences met the Bonferroni-corrected significance threshold for pairwise comparisons (SCH-CTRL: p = 7.6e-10; SCH-BD: p = 1.21e-3; BD-CTRL: p = 0.019; Fig. 2).

**TCN1 association.** To exclude the potentially confounding effects of other variables, we performed a final regression analysis in which we controlled for medication use, diagnosis, education, and vitamin B12 serum levels in addition to age and sex. After adjusting for these confounders, the TCN1 marker was still nominally significantly associated with both verbal learning (β = −3.65, p = 2.60e-3, 95% CI [−6.01, −1.28]) and long delay free recall (β = −1.24, p = 4.05e-4, 95% CI [−1.92, −0.56]). Both the effect size of, and the variance explained by,
TCN1 expression were comparable to that of age, sex, education, and psychiatric disease, all of which are variables that are known to influence memory function (Table 3).

The relationship between TCN1 expression and three subcategories of declarative memory was investigated in order to identify which memory process is the most affected by TCN1 expression. The standardized effect size and the p-value of recognition memory were almost identical to those of verbal learning (CVLT1), indicating that recognition memory along with long-term memory (CVLT2) are the memory domains that are most affected by TCN1 expression (see Supplementary Table S1).

The same two models (initial and final) were used to examine the association between TCN1 expression and verbal learning in the replication sample using HVLT performances converted to z-scores as the memory measure. Despite the change in memory metric, a significant negative correlation was found when age and sex were adjusted for (β = −0.0031, p = 0.0015, 95% CI [−0.0050, −0.0012]). In the final HVLT analysis with adjustment for multiple variables, a negative effect of TCN1 expression with a p-value just above the significance level was found (β = −0.0018, p = 0.062, 95% CI [−3.64e-3, 8.64e-5]). Although the effect sizes in the CVLT and HVLT models were not comparable in magnitude, the direction was the same for both tests and the variance explained by the two models was similar (see Supplementary Table S2).

**Verification of TCN1 microarray measurements.** We verified the microarray-based TCN1 expression data by comparing them to qPCR measurements. qPCR is an orthogonal technology which is considered the golden standard for differential expression profiling. The two methods showed a good overall concordance (n = 82, Pearson’s R = 0.816, 95% CI: 0.73, 0.88; see Supplementary Figure S2), and the strength of correlation was comparable to that of previous studies.23–25.

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**Figure 2.** Pairwise Comparisons of TCN1 Expression across Diagnostic Categories. Expression of TCN1 (F(2, 751) = 20.87, p = 1.5e-9) was significantly different between diagnostic groups. BD: bipolar disorder, CTRL: healthy controls, SCH: schizophrenia. *p > 0.05. **p < 0.001.

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| Predictor | CVLT1 | CVLT2 |
|-----------|-------|-------|
|           | β     | std β | Partial R² | p-value | β     | std β | Partial R² | p-value |
| TCN1      | −3.65 | −1.67 | 0.024      | .0026   | −1.24 | −0.57 | 0.033      | 4.05e-4 |
| Age       | −0.27 | −2.83 | 0.058      | 2.30e-6 | −0.058 | −0.61 | 0.033      | 3.99e-4 |
| Sex (male)| −4.23 | −2.11 | 0.036      | 2.07e-4 | −0.97 | −0.48 | 0.023      | 0.0031  |
| Education (years) | 0.75 | 2.10 | 0.039      | 5.09e-4 | 0.19 | 0.54 | 0.030      | 0.0018  |
| Vitamin B12 | −0.0074 | −0.96 | 2.0e-3 | 0.088 | −0.0014 | −0.18 | 2.4e-4 | 0.27 |
| Medication use (yes) | −1.68 | −0.83 | 2.2e-3 | 0.36 | 0.26 | 0.13 | 6.5e-4 | 0.62 |
| Psychiatric illness (yes) | −3.43 | −1.62 | 8.1e-3 | 0.082 | −1.66 | −0.78 | 0.022 | 0.0039  |

Table 3. Regression Coefficients of Predictor Variables in Final CVLT Models. Standardized β values were calculated after converting all predictor variables to z-scores. CVLT1: Verbal learning score. CVLT2: Long delay free recall score. Std β: Standardized beta coefficients. *p < 0.01.
Expression Quantitative Trait Loci (eQTL) analysis. We examined 127 TCN1-related SNPs for associations with TCN1 expression and found that 6 were nominally significant at the 0.05 significance level. However, none of these SNPs survived correction for multiple testing (see Supplementary Table S3, Supplementary Figures S3, and S4).

Discussion

In the present study, we performed a genome-wide expression analysis to examine the relationship between gene expression in peripheral blood and verbal declarative memory, using the verbal learning (CVLT1) and long delay free recall (CVLT2) measures from the updated California Verbal Learning Test. We identified two genes that were significantly related to memory performance. The first gene, HLA-DRA, was positively correlated with the verbal learning measure. HLA-DRA (Major Histocompatibility Complex, Class II, DR Alpha) is located within the Major Histocompatibility Complex (MHC) locus on chromosome 6 and plays an important role in immune regulation by presenting antigenic peptides to T cells. The MHC region is one of the strongest and most significant susceptibility loci for schizophrenia, and the locus has also been implicated in declarative memory impairment. The second identified gene was TCN1, which was significantly associated with long delay free recall performance and among the top ten genetic markers associated with verbal learning. TCN1 expression was negatively correlated with both measures. This inverse correlation is consistent with the fact that TCN1 is able to bind circulating vitamin B12 but unable to mediate cellular uptake, and with the well-known effect of vitamin B12 deficiency on memory performance.

TCN1’s Role in Cobalamin Transportation. TCN1 (transcobalamin 1; the protein is more commonly referred to as haptocorrin) encodes one of three vitamin B12-binding proteins and is expressed in multiple tissues and secretions. An intricate multistep process is required for the transportation of vitamin B12 (hereafter referred to as cobalamin) through the gastrointestinal tract and the subsequent uptake into the various tissues of the body (Fig. 3A). In the blood stream, cobalamin is bound to either haptocorrin (HC) or transcobalamin (TC; encoded by the gene TCN2) (Fig. 3B). HC is almost fully saturated with cobalamin and binds ~80% of the vitamin with very high affinity, whereas the remainder is bound by TC (holoTC) with higher specificity. However, only the smaller fraction of TC-bound cobalamin, so-called active cobalamin, is available for cellular uptake. The physiological function of circulating HC is not fully understood, but its distinctive ability to bind enzymatically inactive forms of the vitamin with equally high efficiency suggests a role in cobalamin storage and the scavenging of inhibitory cobalamin derivatives.

Cobalamin Metabolism and Memory Performance. The central role of cobalamin in important metabolic pathways is well established. Once taken up by the body’s cells, cobalamin acts as an essential cofactor for two enzymes: methionine synthase, which catalyzes the synthesis of methionine from homocysteine in the cytosol, and methylmalonyl-CoA mutase, which catalyzes the mitochondrial conversion of methylmalonyl-CoA to succinyl-CoA (Fig. 3C). These enzymatic reactions are necessary for the biosynthesis of several molecules that are indispensable for the normal functioning of the central nervous system, including nucleotides, myelin phospholipids, and neurotransmitters. The involvement of cobalamin in these biosynthetic pathways may therefore partly explain the diversity of clinical and neurological manifestations of cobalamin-related deficiencies. Of particular relevance, reduced cellular uptake of cobalamin is associated with psychotic symptoms, depression, mania, cognitive impairment, and memory loss. The association between cobalamin deficiency and memory loss is especially interesting, as individuals with low-normal cobalamin levels have significantly lower hippocampal microstructure integrity compared to individuals in the normal-high range.

Thus, TCN1’s role in cobalamin metabolism and the known effect of cobalamin deficiency on memory function provide a plausible and clinically relevant biological mechanism for the inverse correlation between TCN1 expression and memory performance in a cobalamin-sufficient context (the majority of the subjects in the discovery sample had sufficient cobalamin levels; see Supplementary Table S6). When HC levels in plasma are high, a larger fraction of circulating cobalamin is bound by HC due to its superior affinity in comparison to TC. Consequently, cellular absorption of active cobalamin is reduced, leading to the disruption of metabolic pathways that involve the production of essential chemical compounds, negatively affecting memory function. Interestingly, the pattern of differential TCN1 expression with respect to diagnostic status is also consistent with the proposed mechanism. In our sample, expression of TCN1 gradually increased going from healthy controls to BP patients to SCH patients (Fig. 2). This pattern is in accordance with the well-established finding that cognitive deficits are generally more prominent in schizophrenia than in bipolar disorder. Our results suggest that transcriptional dysregulation of TCN1 may be a contributing factor to the cognitive impairments associated with severe mental disorders.

TCN1 an Important Marker of Memory Performance. Confounding factors are a consistent source of uncertainty in clinical studies. An important strength of our methodological design was the adjustment for critical variables that are either known to influence both gene expression and cognitive performance, such as age, sex, and medication use; or are potential confounders due to their causal associations with either of the predictor or response variables, such as educational attainment, mental disorders, and cobalamin serum levels. By controlling for these factors, we were able to largely exclude the possible effects of secondary contributors. Considering the significant TCN1 correlation in our final analysis where we control for mental disease (Table 3), it does not seem that diagnostic status is the main driver of the negative effect of TCN1 expression on memory function. This is also indicated by the fact that we obtained similar results when performing stratified analyses in which each diagnostic subgroup was examined separately (see Supplementary Table S4 and Supplementary Figure S5). Furthermore, the magnitude of the TCN1 effect on memory performance was comparable to that of sex, age, education, and mental disease (Table 3), suggesting that TCN1 is an important and clinically relevant marker of memory-related functions.
Measurement of Cobalamin in Serum. It is well established that only holoTC, the smaller fraction of circulating cobalamin bound to TC, is available for cellular utility. Consequently, it was suggested three decades ago that serum levels of holoTC could serve as an optimal marker for cobalamin deficiency. At the time, the uncertainty in holoTC measurements was too large to be useful in clinical practice. Subsequent research deploying more sensitive methods, however, has confirmed the soundness of this suggestion. In addition, studies examining the relationship between total serum cobalamin and memory impairment have yielded conflicting results.
results, indirectly confirming the clinical and diagnostic advantage of utilizing holoTC\textsuperscript{52-65}. Other indicators of cobalamin status include homocysteine and methylmalonic acid (MMA), both of which are essential constituents of cobalamin-related metabolic pathways. Although measurement of these metabolites appears to be as sensitive as holoTC, a drawback of both of them is poor specificity\textsuperscript{568}. Despite the apparent advantages of measuring holoTC, there seems to be a general reluctance to begin using the test in both clinical and research practice. Our finding that \textit{TCN1} has a negative effect in individuals that are cobalamin sufficient further strengthens the case for measuring holoTC rather than total cobalamin in both clinical and research applications.

**Blood as a Relevant Tissue for Neurocognitive Function and Disorders.** Since gene expression is tissue-specific, it is generally assumed that only brain tissue is directly relevant for the transcriptional examination of neurocognitive function and mental disease. However, researchers are often compelled to use peripheral gene expression as a surrogate for brain expression due to the limited accessibility and the ethical considerations involved in acquiring human brain samples. This is accepted practice because whole blood shares significant transcriptional similarities with multiple tissues in the central nervous system\textsuperscript{67}. This was in fact the logic behind the design of the present study, but based on our findings it is interesting to speculate whether peripheral blood itself could be a partly relevant tissue for expressionally modeling of cognitive functions and psychiatric diseases. Both \textit{HLA-DRA} and \textit{TCN1} are highly expressed in whole blood, while the mRNA levels in brain are very low or non-existent (www.gtexportal.org), suggesting that the use of blood in addition to brain tissue could be important in order to achieve a complete transcriptomic profiling of brain disorders. Given that immune-related loci are among the most significant and persistent findings in schizophrenia\textsuperscript{88}, it appears that whole blood is a relevant tissue when exploring the expression status of at least a subset of disease-linked genes.

**Limitations of the Present Study.** The clinical interpretation of our findings relies on the assumption that there is a positive correlation between serum mRNA levels and total serum protein concentrations of a given gene. Although this assumption is generally accepted, it does not always hold. In the case of \textit{TCN1}, results should be interpreted cautiously even if the assumption holds since only the smaller fraction of circulating HC which is bound to cobalamin (holoHC; Fig. 3C) can influence cobalamin metabolism. For this reason, further studies aimed at holoTC and holoHC measurements and association with memory performance are highly warranted.

In the replication analysis of \textit{TCN1}, we used HVLT as the verbal learning measure for the majority of study participants. Although the verbal learning measure of HVLT has been shown to be significantly related to the corresponding measure in CVLT\textsuperscript{68} with a relatively high correlation ($r = 0.74$), comparisons between the two tests should be made with caution. This is because the list of words in HVLT is shorter compared to CVLT, and the test may therefore be less sensitive to detect memory decline than CVLT\textsuperscript{68}. The fact that we obtained positive replication results despite this limitation could be an indication of the robustness of our primary \textit{TCN1} finding.

**Conclusions**

In summary, the present study identifies \textit{TCN1} (haptocorrin) as a novel genetic finding associated with poor declarative memory function. The effect size of \textit{TCN1} expression on memory performance is comparable to that of age, sex, and mental illness, all of which are known determinants of memory function. This implies that the role played by \textit{TCN1} could be of clinical importance and relevance. The present findings also suggest that the transcriptional status of this gene could have a diagnostic potential in the assessment of conditions related to cobalamin metabolism. Furthermore, the expression pattern of \textit{TCN1} across diagnostic categories suggests that the cognitive impairments associated with SCH and BD may partly be attributed to changes in \textit{TCN1} expression levels. Finally, our results underscore the clinical importance of using holoTC as a biomarker for cobalamin status rather than total cobalamin.

**Methods**

**Sample characteristics.** The discovery sample ($n = 754$) consisted of 229 healthy controls (CTRL), 234 bipolar disorder patients (BD; 152 type I, 66 type II, 16 not otherwise specified), and 291 schizophrenia spectrum patients (SCH; 212 schizophrenia, 56 schizoaffective, 23 schizophreniform; see Supplementary Table S6). The replication sample ($n = 578$) consisted of 316 CTRL, 67 BD patients (62 type I and 5 not otherwise specified), and 195 SCH patients (142 schizophrenia, 35 schizoaffective, 18 schizophreniform; see Supplementary Table S7). All patients were diagnosed according to the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I)\textsuperscript{99}. Individuals in the CTRL group were excluded if they or their close relatives had a lifetime history of severe psychiatric disorder. All participants gave written informed consent and the study was approved by the Norwegian Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All procedures and methods were carried out in accordance with relevant guidelines and regulations. The recruitment procedure and clinical evaluation for both samples are described in detail in previous reports\textsuperscript{10,11}.  

**Neurocognitive assessment.** Neurocognitive assessment of all study participants was carried out by psychologists trained in standardized neuropsychological testing. The three-hour test battery was administered in a fixed order with two breaks, and included the updated California Verbal Memory Test (CVLT-II) with subscore measures of verbal learning (CVLT1) and long delay free recall (CVLT2)\textsuperscript{72}. Participants were read a list of 16 words in fixed order over five learning trials. After each trial, they were asked to recall, in any order, as many words as they could remember (verbal learning). They were also asked to recall the words after 20 minutes (long delay free recall). The performance metrics used in this study were the sum of scores across the five learning trials (CVLT1) and the long delay measure (CVLT2). The majority of subjects in the replication sample ($n = 465$) were not assessed with CVLT-II, but with the highly similar Hopkins Verbal Learning Test (HVLT)\textsuperscript{73}. In this test, participants were read a list of 12 words over three learning trials. The sum of scores was used as the verbal learning performance metric in the replication analyses.
RNA microarray analysis and quality control. Blood samples were collected in Tempus Blood RNA Tubes (Life Technologies Corporation). Total RNA was extracted with the TEMPSUS 12-Port RNA Isolation Kit (Applied Biosystems) and ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems) according to manufacturer’s protocol. Global gene expression analyses were performed with Illumina HumanHT-12 v4 Expression BeadChip (Illumina, Inc.) consisting of ~47,000 probes. Multidimensional scaling and hierarchical clustering were used for regular quality control, including sample quality measurements and removal of outliers, as well as removal of multiple batch effects (RNA extraction batch, RNA extraction method, DNase treatment batch, cRNA labelling batch, and chip hybridization). This was followed by log2-transformation. More details on microarray preprocessing and quality control are provided in the online supplementary material. Probes showing zero expression in more than 90% of the samples were ignored, leaving 23,476 markers left for examination. All genome-wide expression analyses were performed on the batch-adjusted log2-transformed data.

Validation of microarray data with qPCR. Gene expression data from the microarray measurement of TCN1 were validated with quantitative real-time PCR (qPCR). From the discovery sample, 94 individuals representing all diagnostic categories and spanning the full range of microarray expression levels were picked out for validation. High-Capacity cDNA Reverse Transcription Kit (Life Technologies Corporation) was used for reverse transcription of 1 μg RNA. The qPCR reaction was performed on 5 ng cDNA using TCN1 TaqMan Gene Expression Assay (Hs01055542_m1; Life Technologies Corporation) as target. All samples were run in four replicates. 12 individuals were excluded due to poor cDNA quality. The results were analyzed with qbase+ software (Biogazelle, Zwijnaarde, Belgium) using the comparative cycling threshold (ΔΔCT) method30 with GUSB (Hs00939627_m1) as endogenous control and a positive cDNA control sample as reference. The qPCR measures of TCN1 expression were regressed against the log2 microarray TCN1 gene expression values using a linear model in R 3.4.1.

Expression Quantitative Trait Loci (eQTL) analysis. Subjects in the discovery sample were genotyped at Expression Analysis Inc. (Durham, USA) using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix Inc., Santa Clara, CA, USA). Samples were excluded if they were low-yield (call rate below 97%); if they were duplicates of other samples included in the study; if they were determined to be A autism spectrum disorder and B factor in the microarray analysis. Since TCN1 was among the top ten genes associated with CVLT1 performance and the only gene significantly correlated with the CVLT2 measure, as well as being a biologically and clinically interesting candidate, we explored the effect of potential confounders on the TCN1 association. We performed a final multiple linear regression analysis in which we adjusted for age, sex, diagnosis, medication (categorized as yes or no), and education (total number of years). As TCN1 is known to play an important role in the transportation and uptake of B vitamin B12, we also controlled for total B12 serum levels. We did not adjust for multiple testing in this final analysis. Normality of the variables was checked with the plot() function in R 3.4.1.

To determine which memory process is most affected by TCN1 expression, we examine the relationship between TCN1 expression and three subcategories of declarative memory: working memory, as measured by the first learning trial of CVLT; memory consolidation (or retention rate), as calculated by dividing the long delay free recall (CVLT2) score by the last learning trial; and recognition memory, which is a separate measure within the first learning trial of CVLT; and verbal learning was further examined in the independent replication sample (n = 578) drawn from the same geographical region. The majority of these subjects (n = 465) where not assessed with CVLT but with the highly similar Hopkins Verbal Learning Test (HVLT). Thus, both HVLT and CVLT were used as the verbal learning measure after converting both test values into z-scores. The same covariates as in the main analysis were included in the replication test. All statistical analyses were performed in R 3.4.1.

Data availability. The datasets generated and analyzed during the current study are not publicly available due to Institutional Review Board restrictions but are available from the corresponding author on reasonable request.

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**Author Contributions**

S.D., T.H. and I.A.A. designed the study. V.M.S., O.A.A. and T.U. provided data and/or analytical support. I.A.A. performed all statistical analysis and the quantitative qPCR validation of microarray measurements of TCN1 expression. H.R.B. conducted the microarray experiments. T.U., S.D., V.M.S., O.A.A. and I.A.A. interpreted the results. I.A.A. wrote the manuscript and prepared the tables and figures. All authors reviewed the manuscript.
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