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Single Nucleotide Polymorphisms in ANKK1 and the Dopamine D2 Receptor Gene Affect Cognitive Outcome Shortly After Traumatic Brain Injury: A Replication and Extension Study

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Abstract

Objective—The two objectives of this study were (1) to replicate the previous finding that a single nucleotide polymorphism (SNP) in the ANKK1 gene (SNP rs1800497 formerly known as the DRD2 TAQ1 A allele) is associated with measures of learning and response latency after traumatic brain injury (TBI), and (2) to further characterize the genetic basis of the effect by testing the strength of association and degree of linkage disequilibrium between the cognitive outcome measures and a selected ensemble of 31 polymorphisms from three adjacent genes in the region of rs 1800497.

Method—A cohort of 54 patients with TBI and 21 comparison subjects were genotyped for the DRD2 TAQ1 A polymorphism (rs1800497). Ninety-three patients with TBI and 48 comparison subjects (the current cohort and an earlier independent cohort) were also genotyped for 31 additional neighboring polymorphisms in NCAM, ANKK1, and DRD2. TBI patients were studied one month after injury. All subjects completed memory and attention tests, including the California Verbal Learning Test (CVLT) recognition task and the Gordon Continuous Performance Test (CPT).

Results—As in our previous study the T allele of TAQ1 A (rs 1800497) was associated with poorer performance on the CVLT recognition trial in both TBI and control subjects. There was also a significant diagnosis-by-allele interaction on CPT measures of response latency largely driven by slower performance in the TBI participants with the T allele. Analysis of 31 additional neighboring polymorphisms from NCAM, ANKK1, and DRD2 in the TBI patients showed four haploblocks. A haploblock of 3 SNPs in ANKK1 (rs11604671, rs4938016, and rs1800497 [TAQ1A]) showed the greatest association with cognitive outcome measures.

Conclusions—Our results confirm our previously published association between the TAQ1 A (rs1800497) T allele and cognitive outcome measures one month after TBI, and suggest that a

haploblock of polymorphisms in ANKK1, rather than the adjacent DRD2 gene, has the highest association with these measures after TBI.

Keywords

Traumatic brain injury; polymorphisms; dopamine receptor; cognition

Introduction

Cognitive deficits are the most common source of disability following traumatic brain injury (TBI) [1]. However, the factors influencing cognitive outcome are poorly understood. One can see very different cognitive and functional outcomes following similar injuries in individuals with similar pre-injury intellectual and educational backgrounds suggesting that individual differences such as genotype may play important roles in modulating outcome. Genes that influence neurotransmitters critical to proper cognitive functioning are attractive candidates in this regard. In particular, because of the role that dopamine plays in modulating memory, attention, and frontal-executive functions [2], genes influencing central dopaminergic function are of interest.

The dopamine D2 receptor (DRD2) gene is located on chromosome 11q23 adjacent to NCAM1 and ANKK1, and polymorphisms in these genes are in linkage disequilibrium with each other. All three of these genes have several polymorphisms with fairly high minor allele frequencies. One of these polymorphisms, TAQ1 A (henceforth referred to as rs1800497) has been the focus of significant study [3]. rs1800497 is a C/T single nucleotide polymorphism (SNP) and the presence of the T allele has been associated with a 40% reduction in the expression of D2 receptors in the striatum and perhaps other cortical regions, without change in receptor affinity [4,5]. Originally regarded as a DRD2 polymorphism, rs1800497 was subsequently recognized to be a functional coding polymorphism in the adjacent gene, ANKK1. This raises the question whether this SNP is in linkage disequilibrium with some other functional DRD2 polymorphism or perhaps regulates DRD2 levels in some as yet unclear fashion [6].

We previously examined the effect of rs1800497 on cognition after TBI [7]. In our original study the T allele was associated with poorer performance on a measure of episodic memory (the California Verbal Learning Test - recognition trial), and there was a significant diagnosis-by-allele interaction on measures of response latency (Gordon Continuous Performance Test) whereby subjects with TBI and the T allele had the worst performance in a cohort of 39 patients with mild TBI and 27 healthy controls. An association between a single nucleotide polymorphism and measures of cognitive outcome is a provocative finding. However it is critical to replicate this relationship before drawing firm conclusions about this. The advent of high throughput genotyping technologies has enabled large numbers of polymorphisms (within a given gene, across many genes, or genome-wide) to be examined for association with a given outcome measure. This greatly increasing the risk of a Type 1 (false positive) error. For example Hirschhorn et al. (2002) reviewed 600 such genetic association studies and found that only 27% had been studied three or more times. Of these only about 4% were consistently reported. Thus efforts to replicate previous associations are critical to our efforts to understand the genetic contribution to such complex clinical states such as cognitive outcome after TBI..

The objective of this study was to replicate our previous findings in an independent cohort, and to clarify whether rs1800497 affects cognitive outcome itself, or is a marker for one or more other SNPs in neighboring regions. Replication of this finding from a new independent sample of individuals with TBI from our center would not definitively address the

generalizability of the results to other populations. However it would provide a firmer foundation on which to explore such studies in other groups of people with TBI. We hypothesized that (a) rs1800497 would be associated with cognitive measures shortly after TBI in the independent cohort and (b) that rs1800497 is in linkage disequilibrium with a functional polymorphism in the promoter region of the DRD2 gene and thus polymorphisms in this region would show stronger associations with the cognitive measures than rs 1800497.

Methods

This study consisted of two parts. In the initial phase we attempted to replicate, our original finding of an association between rs1800497 and cognitive outcome measures after TBI in an independent cohort of TBI patients and healthy controls. In the second phase we combined our two cohorts and tested the strength of association between the cognitive outcome measures and an ensemble of 31 carefully selected candidate polymorphisms from three adjacent genes: NCAM1, ANKK1, and DRD2. Each polymorphism was tested individually and in combination with other polymorphisms (see below). Methods for each phase are described separately.

Replication Phase

Participants—A cohort of 54 consecutive patients with mild – moderate TBI (5) were recruited from a Level-1 trauma centre emergency department. Mild-moderate TBI was defined as initial Glasgow Coma Scale scores of 9–15 when available and/or duration of loss of unconsciousness not exceeding 24 hours. The American Congress of Rehabilitative Medicine criteria for mild TBI was used as the minimum standard for having sustained a brain injury. TBI participants were studied approximately one month (mean=43.1 days, SD =15.8) after their injury. Healthy control subjects (n=21) were recruited through advertisements. Exclusion criteria included history of other neurological disorders, substantial systemic medical illness, or current DSM-IV axis I psychiatric diagnosis (based on the Structured Clinical Interview for DSM-IV [8]) with the exception of substance abuse. The study protocol and informed consent were approved by the Dartmouth College Committee for the Protection of Human Subjects. Written informed consent was obtained from all participating subjects. Over 99% of both the TBI and control groups were Caucasians of European descent.

Cognitive Measures—Participants were given the Wide-Range Achievement Test, Third edition, (WRAT-3) Reading subtest [9], the WAIS-III Block Design subtest [10], the California Verbal Learning Test (CVLT) [11,12], and the Gordon Continuous Performance Test (CPT)[13].

Genotyping—QIAGEN's blood mini kit (QIAGEN, Alameda, CA.) was used to isolate DNA from peripheral blood samples. rs 1800497 allele status was determined by using real-time polymerase chain reaction and Eclipse MGB probes (Epoch Biosciences, Bothell, WA) as described in our previous report [7].

Extension Phase

Participants—For this analysis the TBI subjects and healthy controls from the original cohort [7] and the independent cohort described above were combined for a total of 93 individuals with TBI and 48 healthy controls. The selection and exclusion criteria for participants, post-injury assessment period and cognitive assessment measures were the same for the two independent samples. Mean injury to assessment interval for the TBI subjects in the combined sample was 40.2 days (SD=16.2).

Genotyping—In addition to the “TaqA” polymorphism (rs1800497) genotyped as part of the replication phase of this study, thirty additional polymorphisms in DRD2, NCAM1 and ANKK1, were genotyped and tested for association with cognitive measures.

The DRD2 -141 ins/del promoter polymorphism (rs1799732) was genotyped using the 5'nuclease (“TaqMan”) assay described by Gemignani et al[14] and implemented on the ABI 7500 FAST system. For all other polymorphisms genotyping was done using the Dartmouth Neurochip, a 3300 SNP microarray gene chip developed in partnership between the Dartmouth Neurogenetics Group (DNG) and Affymetrix (Affymetrix Inc., Santa Clara, CA). Candidate genes and alleles were selected for inclusion on the chip after extensive deliberation by the DNG in the context of review of the available human and animal literature, making use of multiple databases of genetic associations with neurological, psychiatric, neurodevelopmental and neurodegenerative disorders of the CNS and relevant phenotypic markers. Selection criteria included known or hypothesized involvement in critical pathways involved in cognition, neurotrauma, neurodegeneration, excitotoxic and injury cascades, neural plasticity and repair, inflammation, immune responsivity, stress response/reactivity, and depression/anxiety. Criteria used to assign relative importance to candidate genes/alleles included; (1) having a probable role in modulation of the above processes, (2) having minor allele frequencies of at least 5%, and (3) known functional effects or located in gene regions that might impact on gene expression (e.g., promoter regions). The resulting array was implemented by Affymetrix using molecular inversion probe technology and consisted of 3300 single nucleotide polymorphisms (SNPs) from approximately 1000 candidate genes. Ancestral informative markers were also included to examine population substructure. We have successfully obtained genotype data on an initial sample of 1,000 individuals with several neuropsychiatric disorders including TBI. Reliability and quality control were excellent. For this study we looked only at SNPs selected in the region of rs1800497.

Genetic Analyses—Bayesian clustering as implemented in the program Structure [15] was used to look for significant genetic subpopulations within our sample. This analysis showed no differences in population structure between the TBI participants and healthy controls.

Minor allele frequencies were estimated and used along with estimated genotype frequencies to test for departures from Hardy-Weinberg equilibrium (HWE). Based on both the chi-square and exact test analyses of HWE, all polymorphisms but one (rs7131056) had genotype frequencies that were consistent with HWE proportions. We chose to leave this SNP in the analysis. This SNP was not significantly correlated with cognitive outcome in TBI patients ($p=0.67$) or healthy controls ($p=0.24$), and its frequency did not differ between groups ($X^2 = 4.44$, $df = 2$, $p = 0.11$). Except for one monomorphic SNP and one with a minor allele frequency of 0.009, all SNPs had a minor allele frequency > 0.1 , which is frequently used as a cutoff for common alleles.

The extent of linkage disequilibrium (r^2) between each pairwise combination of SNPs in the control subjects was estimated and visualized using Haploview [16]. The default setting in Haploview highlighted four regions of high linkage disequilibrium (LD) in the three-gene region (Figure 1).

Statistical Analyses—Between-group differences in demographic characteristics were examined by t-tests or chi-square tests for continuous or categorical variables respectively. Group differences in cognitive performance related to rs1800497 T allele status and haplotype status were examined by linear regression and ANOVA with Tukey's HSD corrections for multiple comparisons. [17,18]

Polymorphism associations—Each of the 31 allele variables was summarized as either a factor variable with three levels (0,1,2), or as a genotype variable representing dominant, recessive, or additive models. Group differences for cognitive measures were analyzed by ANCOVA with diagnosis, the allele variable and the interaction between allele and diagnosis included in the model, covarying for age and education. Significance tests were examined for the main effects of diagnosis and allele status, and for the interaction between allele and diagnosis. p-values were adjusted for multiple comparisons using the false discovery rate (FDR) method of Benjamini and Hochberg [19], with each p-value characterized as to whether it was significant with respect to an FDR of 0.05, 0.10, or 0.20. For both studies two-tailed p-values were used for significance thresholds.

Haplotype associations—Haplotype trend regressions (HTR) were performed in the combined TBI sample using PowerMarker [20] with a 3- and 4-SNP sliding window. Haplotypes and their frequencies were estimated with the expectation-maximization (EM) algorithm.[21,22]. The association of each estimated haplotype with the cognitive variables of interest were examined through HTR using the gap package in R (<http://www.lmbe.seu.edu.cn/CRAN/src/contrib/Descriptions/gap.html>)[23]. Linear regression was also used to confirm the results from the HTR.

Results

Replication Phase

Demographics—Demographic and injury variables are summarized in Table 1. The TBI group was slightly older ($t = -2.23$, $p < .03$) than the control sample. There were no significant between-group differences in gender, education or parental education. There were also no between-group differences in baseline intellectual function based on WRAT-3 reading score or WAIS-III Block Design raw score. Age and education were used as co-variables for subsequent analyses.

Genotype Distribution—With respect to genotype at rs1800497, there were 49 C/C (homozygous for the C allele), 25 C/T(heterozygotes), and 1 T/T (homozygous for the T allele) participants in the combined group of TBI and control subjects. The distribution of T allele was similar in the two groups (TBI=17 [32%], controls=9 [43%]; $X^2 = 1.482$, $p = 0.48$). Within the TBI group, 37 individuals were T-allele-negative, and 17 were T-allele-positive. Comparison of T-allele-negative and T-allele-positive TBI subjects revealed no between-group differences in age, gender, education, parental education, WRAT-3 reading or WAIS-III Block Design scores, number of days tested post-injury, or injury severity as assessed by Glasgow Coma Scale score (14.4 in the T-allele-negative group vs. 13.8 in T-allele-positive group; $t = 1.22$, $p = 0.23$).

Effects of Genotype on Cognition—There was a trend for a main effect of allele type on CVLT recognition trial performance across all subjects (T allele present: mean=11.6, SD=3.7, versus T allele absent: mean=12.9, SD=3.0; $F=2.64$, $p=0.10$). There was a significant main effect of diagnosis (TBI versus control) on CPT response latency measures (simple reaction time [$F=6.88$, $p=0.01$], vigilance [$F=14.37$, $p=.0003$], and distractibility [$F=8.66$, $p=0.004$]), with TBI subjects showing slower response times across trials. There was also a significant diagnosis (TBI versus control)-by-allele (T-allele-positive versus T-allele-negative) interaction for the Vigilance and Distractibility conditions of the Continuous Performance Test. As can be seen in Figure 2, T-allele-positive TBI subjects had slower response latencies compared to T-allele-negative TBI subjects, and slower response latencies than the controls regardless of genotype.

Linear regression including an interaction term for the original and replication studies showed no significant interactions between the original and replication studies (data not shown). This provides further support for similarity of the directionality of the effect in these two independent cohorts.

Extension Phase

For the extension phase we combined the original cohort [7] with the current cohort and looked for the strength of association between cognitive outcome, rs1800497 allele status, and allele status in 30 neighboring polymorphisms.

Demographics—The combined cohort included in 93 individuals with TBI and 48 control subjects. Demographic and injury variables are summarized in Table 2. There were no significant between-group differences in gender or parental education, although there was a difference in years of education, with the control group being slightly more educated ($t=2.17$, $p=.03$). There was a statistical difference on one of the measures of baseline intellectual function (WRAT-3 reading score; controls: 109.4 vs. TBI: 105.6); however, this is not considered a clinically meaningful difference and there was no difference between the groups in terms of performance on WAIS-III Block Design raw score. Age and education were used as covariates for subsequent analyses.

Association with rs1800497 in the Combined Group—With respect to genotype at rs1800497 there were 93 participants homozygous for the C allele (C/C), 47 heterozygotes (C/T) and 1 homozygous for the T allele (T/T) in the combined group of TBI patients and controls. The distribution of T allele was equivalent in the two groups ($X^2 = 2.67$, $p = 0.26$). Within the TBI group, 65 individuals did not have a T allele (were T-allele-negative), and 28 had at least one T allele (were T-allele-positive). There were no significant between-group differences in age, education, parental education, WRAT-3 reading or WAIS-III Block Design scores, number of days tested post-injury, or injury severity as assessed by Glasgow Coma Scale score. There was a significant gender difference, with fewer women in the T-allele-positive group (T-allele-negative: 37 M, 28 F; T-allele-positive: 22 M, 6 F: $X^2 = 3.96$, $p=0.047$).

There was a significant main effect of having a T allele at rs1800497 on CVLT recognition trial performance across all participants in the combined sample: lower performance was associated with presence of the T allele (T allele present: mean=12.6, SD=3.72, versus T allele absent: mean=13.9, SD=2.6; $F=5.29$, $p=0.02$). This effect was also seen in the TBI group alone (T-allele present: mean = 11.52, sd = 4.02, T allele absent: mean = 13.86, sd = 2.30; $p = .007$), but not in the controls alone. A significant interaction between diagnosis and genotype on CVLT recognition performance was also present ($F = 4.29$, $p=0.04$), driven by the effect of the T allele seen in the TBI patients.

There was a significant main effect of diagnosis (TBI versus control) on CPT response latency measures (simple reaction time [$F=14.03$, $p=0.0003$], vigilance [$F=21.42$, $p<0.00001$], and distractibility [$F= 14.51$, $p=0.0002$]), with TBI subjects showing slower response times across trials. There was also a significant diagnosis-by-allele (T-allele-positive versus T-allele-negative) interaction for each of the CPT trials. As can be seen in Figure 3, T-allele-positive TBI subjects had slower response latencies compared to T-allele-negative TBI subjects, and slower response latencies than controls regardless of genotype.

Haplotype analysis—In order to better characterize the nature of the observed genetic effect on cognition following TBI, we examined CVLT recognition trial performance, which demonstrated both a main effect for allele status and an interaction effect, and tested the

strength of association between this measure and the 31 candidate polymorphisms in the combined sample of TBI patients. We initially performed linear regression, adjusting for age and education, to analyze the correlation between the genotype at each SNP. We ran the regression for dominant, recessive, and additive genetic models and selected the lowest p value for each polymorphism (See Table 3).

Eleven polymorphisms had significant p values after correction with a false discovery rate (FDR) of 0.2 (see Table 3) and clustered in areas of high LD. To determine if specific combinations of alleles at these locations (haplotypes) were more predictive of cognitive outcome than any individual SNP, we performed a haplotype trend regression (HTR) on combinations of the polymorphisms using the program PowerMarker [20] and an initial sliding window of 4 polymorphisms. The polymorphism combinations with the lowest p values each contained three polymorphisms located in ANKK1: rs11604671, rs4938016, and rs1800497 (TAQ1 A) (Figure 4a). Using a 3-polymorphism sliding window (Figure 4b), these three polymorphisms resulted in the lowest p value of all the 3 SNP combinations ($p = 0.008$).

As seen in Figures 4a/b, there are some additional peaks that associate with the CVLT recognition performance within the DRD2 gene itself. However these peaks had less significant p values than the window containing rs1800497, and their significance can be attributed to being in high LD with the window containing that SNP (Figure 1).

To investigate whether any of the haplotypes in this region of high LD significantly related to CVLT score, we estimated the haplotypes and their frequencies using the expectation maximization (EM) algorithm in PowerMarker. There were 5 estimated haplotypes, three with frequencies above 0.15 (Table 4). HTR revealed that haplotype 2 (genotype G-G-C; frequency = 0.31) was associated with CVLT recognition performance ($F = 7.30$, $p = 0.008$). Linear regression with haplotype 2 indicated that TBI subjects with haplotype 2 had a significantly higher CVLT recognition score (mean=14.21, SD=2.21) when compared to those without haplotype 2 (mean=12.65, SD=3.66) ($p = 0.006$, $r^2 = 0.06$). Linear regression performed on the rs1800497 T-allele-positive (mean CVLT recognition = 12.64, SD=3.72) and T-allele-negative (mean CVLT recognition = 13.93, SD=2.57) TBI subjects showed a similar but somewhat weaker association ($p = 0.02$, $r^2 = 0.03$) suggesting that there is additional signal from the 3 SNP haplotype over and above the information from rs1800497 alone.

Conclusions

The results from our independent replication cohort are quite similar to those seen in our original cohort and substantiate an association between polymorphisms in the region of rs1800497, reaction time response latencies and verbal recognition memory shortly after mild-moderate TBI. Contrary to our original hypothesis, the haplotype analysis in the expanded sample of TBI patients supports the hypothesis that the functional polymorphism related to these cognitive variables lies within the ANKK1 gene in a region that includes rs1800497, and that the association signal is not simply due to linkage disequilibrium between this locus and a functional polymorphism in DRD2.

There are several limitations of this study that should be noted in interpreting the results. This is a cohort of individuals with predominantly mild injuries studied approximately one month after injury. The results may not be generalizable to more severely injured populations and longer post-injury intervals. Cognitive outcome after TBI is a complex interaction of pre-injury capacities, the profile and type of brain injury, effects of injury to other areas, post-injury treatment and various psychosocial factors. Thus the contribution of

a single polymorphism to outcome is quite modest. In more severely injured individuals or in longer injury to outcome intervals these other contributing factors may overwhelm the contribution of a single polymorphism. This is why we chose to look initially in a cohort of individuals with mild injuries. We are currently looking to see if there are similar effects in more severely injured individuals and if the effects hold true one year after injury. Our cohorts are from a largely northern European Caucasian population reflecting the demographics of our catchment area and thus further work is needed to determine the generalizability to other racial and ethnic populations. Although mildly injured as a group, we cannot exclude the possibility that there are subtle but significant differences in injury severity between the groups that are unrelated to genotype at various SNPs such as rs1800497. It is difficult to ascertain other injury severity indicators such as duration of loss of consciousness (e.g. many of the events are unwitnessed accidents) or duration of post-traumatic amnesia (e.g. self-report cannot be considered reliable in this population, and medical records are often not sufficiently detailed for accurate determination). Although the association signal appears to come from ANKK1, we cannot rule out the possibility that these SNPs are in LD with an as yet undiscovered functional polymorphism in a nearby region of either ANKK1 or DRD2. Nevertheless it is of interest that we have replicated our original finding of an association between polymorphisms in the ANKK1/DRD2 region and indicators of cognitive outcome approximately one month after mild-moderate TBI.

Until 2004 rs1800497 was thought to lie in a regulatory region of DRD2, and indeed, functional data seemed to support this hypothesis. The T allele at that locus has been associated with reduced DRD2 receptor density [24,25] and binding in the human striatum, [4] although these findings are controversial [26]. In 2004 Neville et al. mapped rs1800497 to the last exon in the gene ANKK1 [6]. Little is known about ANKK1, though [6] describe it as a novel member of the serine/threonine kinase gene family with 11 ankyrin repeats. Ankyrin repeats are typically involved in protein-protein interactions and ankyrin repeat proteins are involved in widely diverse processes, including transcription initiation. [27,28]. Members of the serine/threonine kinase family are involved in signal transduction as well as in mediating cellular response to external stimuli. It is conceivable that the ANKK1 gene is involved directly in dopaminergic signaling or in DRD2 transcription. ANKK1 expression has yet to be found in the brain, however, [6] and it is still possible that rs1800497 is in linkage disequilibrium with a functional polymorphism in DRD2. More studies with finer mapping of the DRD2 and ANKK1 genes are needed to further delineate where the functional polymorphisms lie, and studies elucidating the functions of ANKK1 will be helpful in resolving the controversies around rs1800497.

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Figure 1. Linkage disequilibrium (LD) between each pairwise combinations of the 31 SNPs in the DRD2 region. Heavy black lines outline the 4 haploblocks. The numbers in the squares are the measure of linkage disequilibrium, D' . Red squares indicate high LD while blue and white squares represent lower LD. Each SNP lies in the gene blocked out above it. The figure is not to scale, and the SNPs are not evenly spaced. Red lightning bolts between SNPs indicate a large physical gap between SNPs on the chromosome.

Figure 2.

Performance on the Continuous Performance Test as a Function of Diagnostic Group (Healthy Comparison Subjects or Patients with TBI) and rs1800497 T Allele Status (Absent or Present) for the independent cohort. For Simple Reaction Time, there was a significant diagnosis-by-allele interaction ($F=2.31$, $df = 1, 74$, $p = 0.04$). For the Vigilance condition, there was a significant diagnosis-by-allele interaction ($F=5.19$, $df = 1, 74$, $p < 0.003$). For the Distractibility condition, Vigilance, there was a significant diagnosis-by-allele interaction ($F=3.08$, $df = 1, 74$, $p < 0.04$).

The * indicates that the control T-negative patients had significantly shorter reaction times than both groups of TBI patients regardless of genotype ($p < 0.01$).

Figure 3. Performance on the Continuous Performance Test as a Function of Diagnostic Group (Healthy Comparison Subjects or Patients with TBI) and rs1800497 T Allele Status (Absent or Present) for the Combined Sample. For Simple Reaction Time, there was a significant diagnosis-by-allele interaction ($F=2.31$, $df = 1, 74$, $p = 0.04$). For the Vigilance condition, there was a significant diagnosis-by-allele interaction ($F=5.19$, $df = 1, 74$, $p<0.003$). For the Distractibility condition, there was a significant diagnosis-by-allele interaction ($F=3.08$, $df = 1, 74$, $p<0.04$)

a pairwise significant difference with $p < 0.05$

** a pairwise significant difference with $p < 0.01$

† a pairwise significant difference with $p < 0.001$

Figure 4a

Figure 4b

Figure 4. Plot of the $-\log(p \text{ value})$ from the haplotype trend regression in PowerMarker associating a sliding window of SNPs with CVLT Recognition task performance

(a) Results of haplotype trend regression using a sliding window of four SNPs and CVLT Recognition task performance. The horizontal line marks a significance level of 0.05.

(b) Results of haplotype trend regression using a sliding window of three SNPs and CVLT Recognition task performance. The horizontal line marks a significance level of 0.05.

Table 1

Demographic Characteristics of the Replication Sample

	TBI Group (N=54)	Healthy Control Group (N=21)	
Age	35.2 ± 13.6	27.9 ± 10.3	0.028
Sex (M, F)	39 M, 15 F	13 M, 8 F	n.s. (chi-square)
Education	14.4 ± 2.5	15.5 ± 2.5	n.s.
Mother's Education	13.4 ± 2.6	14.5 ± 3.4	n.s.
Father's Education	13.8 ± 3.6	15.0 ± 2.7	n.s.
WRAT Reading Score	106.9 ± 9.3	109.0 ± 6.2	n.s.
Block Design (WAIS-III), Raw Score	50.0 ± 11.9	50.7 ± 9.1	n.s.
Glasgow Coma Scale Score (N=45)	14.1 ± 1.5	N/A	

Table 2

Demographic Characteristics of the Extended Sample

	TBI Group (N=93)	Healthy Control Group (N=48)	
Age	33.8 ± 13.4	30.4 ± 10.1	n.s.
Sex (M, F)	59 M, 34 F	25 M, 23 F	n.s. (chi-square)
Education	14.2 ± 2.6	15.2 ± 2.2	0.03
Mother's Education	13.6 ± 2.6	14.3 ± 3.0	n.s.
Father's Education	14.1 ± 3.6	14.7 ± 3.1	n.s.
WRAT Reading Score	105.6 ± 10.2	109.4 ± 6.8	0.01
Block Design (WAIS-III), Raw Score	49.9 ± 11.9	51.3 ± 8.7	n.s.
Glasgow Coma Scale Score (N=80)	14.2 ± 1.6	N/A	

Table 3

Degree of Association Between 31 SNPs and CVLT Recognition Task Performance in Combined Sample. Shown are the results of the linear regression adjusting for age and education. The regression was run for dominant, recessive, and additive genetic models and the lowest p value was selected for each polymorphism. Values in bold had significant p values after correction with a false discovery rate (FDR) of 0.2

Gene	SNP	p value	
		cases	controls
NCAM1 ANKK1	rs2226849	0.72	0.56
	rs965560	0.39	0.078
	rs2303377	0.78	0.87
	rs877138	0.46	0.88
	rs11604671	0.29	0.22
	rs4938016	0.10	0.16
	TAQ1A (rs1800497)	0.012	0.94
DRD2	rs2234689	0.07	0.77
	rs2242592	0.029	0.13
	rs6278	0.017	0.81
	rs6279	0.028	0.13
	rs6277	0.29	0.13
	rs6275	0.028	0.13
	rs1076560	0.017	0.81
	rs1800498	0.41	0.14
	rs1079597	0.017	0.81
	rs7131440	0.41	0.14
	rs2471851	0.017	0.78
	rs4587762	0.50	0.14
	rs4586205	0.019	0.16
	rs17529477	0.68	0.65
	rs4245147	0.52	0.81
	rs4245148	0.064	0.87
	rs4460839	0.064	0.98
	rs7122454	0.038	0.47
	rs7131056	0.40	0.089
	rs4630328	0.41	0.85
	rs4938019	0.022	0.47
	ins/del	0.054	0.99
	rs1799978	0.62	0.46
	rs12364283	0.51	0.22

Table 4

Haplotypes estimated by the EM algorithm for the complete set of subjects.

Haplotype	Genotype *	Frequency
1	A-C-T	0.51
2	G-G-C	0.31
3	G-C-T	0.18
4	G-C-C	0.004
5	G-G-T	0.004

* Genotypes at nucleotides rs11604671, rs4938016, and rs1800497 (TAQ1 A) respectively.