Association of a common genetic variant within ANKK1 with six-month cognitive performance after traumatic brain injury

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Abstract Genetic association analyses suggest that certain common single nucleotide polymorphisms (SNPs) may adversely impact recovery from traumatic brain injury (TBI). Delineating their causal relationship may aid in development of novel interventions and in identifying patients likely to respond to targeted therapies. We examined the influence of the (C/T) SNP rs1800497 of ANKK1 on post-TBI outcome using data from two prospective multicenter studies: the

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Citicoline Brain Injury Treatment (COBRIT) trial and Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot). We included patients with ANKK1 genotyping results and cognitive outcomes at six months post-TBI (n=492: COBRIT n=272, TRACK-TBI Pilot n=220). Using the California Verbal Learning Test Second Edition (CVLT-II) Trial 1-5 Standard Score, we found a dose-dependent effect for the T allele, with T/T homozygotes scoring lowest on the CVLT-II Trial 1-5 Standard Score (T/T 45.1, C/T 51.1, C/C 52.1, ANOVA, p=0.008). Post hoc testing with multiple comparison-correction indicated that T/T patients performed significantly worse than C/T and C/C patients. Similar effects were observed in a test of non-verbal processing (Wechsler Adult Intelligence Scale, Processing Speed Index). Our findings extend those of previous studies reporting a negative relationship of the ANKK1 T allele with cognitive performance after TBI. In this study, we demonstrate the value of pooling shared clinical, biomarker, and outcome variables from two large datasets applying the NIH TBI Common Data Elements. The results have implications for future multicenter investigations to further elucidate the role of ANKK1 in post-TBI outcome.

Introduction

Traumatic brain injury (TBI) is a complicated injury in a complex organ. Each year in the USA, at least 2.5 million people suffer TBIs. This includes 52,000 deaths, 275,000 hospitalizations, and 1.365 million treated and released from an emergency department (ED) [1]. TBI is a contributing factor to 30 % of all injury-related deaths in the USA [1]. An estimated 3.2 to 5.3 million persons currently live with long-term physical, cognitive, and neuropsychiatric disabilities attributable to TBI [2]. Heterogeneity of the primary injury is complicated by a host of patient-specific factors that together determine clinical outcome [3]. Understanding how physiological factors influence patient outcome provides an avenue for identifying methods of clinical intervention, as well as the patients most likely to benefit. The advent of the Human Genome Project and genetic association analyses has allowed the identification of several polymorphic alleles of candidate genes that may signal disparate outcomes following TBI. However, examination of large numbers of genes results in high chance of type 1 error, underscoring the need for repeat studies of larger samples and high statistical power [4].

Cognitive deficits are among the leading sources of morbidity in TBI patients, and the underlying mechanisms are poorly understood. Patients presenting with similar injuries exhibit disparate patterns of cognitive impairment. The source of this variability is presently unknown but may involve genetic modulation as well as subtle morphometric differences in injury characteristics, highlighting the importance of investigating genetic differences that modulate cognitive function [5]. Previous studies have examined genes that modulate the dopaminergic pathway, which is critical to attention, memory, and executive function. As a result, the dopaminergic system is frequently targeted, through pharmacologic manipulation, to ameliorate chronic deficits in these areas following TBI [6].

Ankyrin repeat and kinase domain-containing 1 (ANKK1) is a candidate gene involved in dopamine transmission [7, 8]. In human adults, ANKK1 mRNA and protein is expressed in the central nervous system (CNS), exclusively in astrocytes [9]. The ANKK1 protein, also known as SgK288, shares structural homology with a family of serine/threonine receptor-interacting protein kinases (RIPKs) potentially responsible for signal transduction and cellular response modulation of dopaminergic reward processes [10].

A common single nucleotide polymorphism (SNP) in the ANKK1 gene may impact outcome after TBI [11, 12]. The C/T SNP rs1800497, also known as Taq1A, is located on chromosome band 11q23.1 in exon 8 of ANKK1 and causes a p. Glu713Lys amino acid change in the C-terminal ankyrin repeat domain, which is involved in protein-protein interaction [10]. Rs1800497 is located 10 kB downstream of the DRD2 gene. While unlikely to directly control DRD2 expression, it may be located within a regulatory region for a functional SNP in the DRD2 gene [10]. Positron emission tomography (PET) studies have shown that rs1800497 affects dopamine binding in the striatum in healthy volunteers [13]. Presence of a single T allele is associated with a 30–40 % reduction of dopamine D2 receptor (DRD2) density in the ventral striatum compared to homozygotes with C alleles, suggesting that T allele carriers may require increased dopaminergic tone to achieve similar levels of reinforcement and reward as C/C individuals. Studies have shown that one or two copies of the T allele of rs1800497 associates with disorders of reward deficiency such as alcohol dependence, smoking, and addictive behavior [14–17]. McAllister et al. found that rs1800497 allele status was associated with cognitive function following mild to moderate TBI (N=141: 93 TBI patients, 48 healthy controls) as defined by initial Glasgow Coma Scale (GCS) score of 9–15 and/or loss of consciousness (LOC) ≤24 h [11, 12]. The TBI group included 65 T-allele negative and 28 T-allele positive patients. T-allele positive patients showed worse episodic memory at 1 month post-TBI on the California Verbal Learning Test (CVLT) recognition trial, a result not observed in controls with the T allele. T-allele positive patients in the TBI group also exhibited slower performance on measures of response latency than those without the T allele [11, 12].

The present study extends this work in evaluating whether variation at rs1800497 within ANKK1 associates with verbal learning and non-verbal learning after acute TBI in a large multicenter cohort. We combined clinical and outcome data from two large prospective multicenter studies, The Citicoline Brain Injury Treatment Trial (COBRIT) [18, 19] and the Transforming Research and Clinical Knowledge in Traumatic Brain Injury Pilot (TRACK-TBI Pilot) [20] to create the largest sample size to date of adult TBI patients with rs1800497 genotyping and six-month outcome testing after acute TBI (N=492). The merging of these two large datasets was made possible by their shared common standards—the National Institute of Neurological Disorders and Stroke (NINDS) TBI Common Data Elements (CDEs) [21]. We tested the primary hypothesis that the rs1800497 associates with reduced performance on the CVLT as previously described by McAllister et al. [11, 12] and assessed secondary endpoints and tertiary endpoints including a measure of non-verbal processing (Wechsler Adult Intelligence Scale Processing Speed Index (WAIS-PSI)).

Materials and methods

Study design

COBRIT is a multicenter, two-group, phase three, double-blind randomized placebo-controlled clinical trial conducted at eight U.S. Level I Trauma Centers [18, 19]. Inclusion criteria were patients with blunt force trauma to the head requiring inpatient hospitalization for TBI, with either: (1) Glasgow Coma Scale (GCS) score 3–12 and GCS motor score <6, or (2) GCS 3–12 with motor score 6 *or* GCS 13–15 *or* paralyzed after administration of paralytics as part of the clinical course with ≥1 of the following CT parameters: ≥10-mm diameter intraparenchymal hemorrhage, ≥5-mm extra-axial hematoma, subarachnoid hemorrhage visible on two or more 5-mm slices, or midline shift ≥5 mm. TRACK-TBI Pilot is a multicenter prospective observational study with patients recruited through convenience sampling at three U.S. Level I Trauma Centers [20]. Inclusion criteria were external force trauma to the head and clinically indicated head CT scan within 24 h of injury.

Exclusion criteria for both studies included positive pregnancy test result or known pregnancy, imminent death or current life-threatening disease, incarceration, or evidence of serious psychiatric and neurologic disorders that interfere with outcome assessment. Non-English speakers were not enrolled due to inability to participate in outcome assessments, which are normed and administered in English. The COBRIT study also excluded patients with bilaterally fixed and dilated pupils, those with prior TBI requiring hospitalization, concurrent enrollment in another study, and/or acetylcholinesterase inhibitor use within two weeks prior to injury. One trauma center (University of Pittsburgh) participated in both COBRIT and TRACK-TBI Pilot, but patients at this site were not co-enrolled into both studies.

The institutional review boards of all participating sites approved the protocols for each study. Patients were approached for informed consent before enrollment. For patients unable to give consent, due to their injury, consent was obtained from their legally authorized representative (LAR). Patients consented by LAR were approached for informed consent to continue participation while in the hospital or during follow-up assessment time-points.

These two studies enrolled a large number of TBI patients through acute and intermediate care to provide an ethnically and demographically diverse patient population. In TRACK-TBI Pilot, a comprehensive acute clinical profile was obtained from each patient in accordance with the National Institutes of Health (NIH) and NINDS CDEs across demographics, medical history, injury characteristics, acute hospital clinical care, and neuroimaging [22–26]. Enrollment in COBRIT began prior to the release of the NIH NINDS CDEs, but variables were collected in a standardized fashion with a high degree of concordance with the CDE effort [18], which enabled data pooling between the two studies. The pharmacological intervention in COBRIT consisted of daily enteral/oral citicoline (2000 mg) or placebo for 90 days. As the primary report by Zafonte et al. in 2012 found no association between citicoline use and improvement in functional and cognitive outcome [19], we did not pursue outcome analysis between treatment and control arms for this study.

Patient selection

All adult patients with complete 6-month outcomes and an acute blood biospecimen drawn for DNA were selected for this analysis from the COBRIT and TRACK-TBI Pilot studies. In both studies, patients without genotyping results and/or complete 6-month outcomes were excluded. Of 1213 total adult patients in COBRIT, 739 patients did not have blood genotyping results available and 202 of the remaining 474 patients had no or incomplete outcomes, leaving a final N of 272 patients for analysis. Of 650 total patients in TRACK-TBI Pilot, 51 patients were excluded from the non-acute TBI site and 27 patients were under the age of 18. Of the remaining 572 adult patients, 166 did not have blood genotyping results and 186 had genotyping but no or incomplete outcomes, leaving a final N of 220 for analysis. A comparison between included and excluded adult patients for this analysis, by study, is discussed in the Results section and in Online Resource 1 and 2. The distributions of demographic and clinical descriptors for COBRIT patients by treatment group are summarized in Online Resource 3.

Blood collection and genotyping

Specimen acquisition was performed as previously described [20]. In brief, blood samples for DNA genotyping analysis were collected via peripheral venipuncture or existing peripheral venous indwelling catheters within 24 h of injury. Samples were collected in BD Vacutainer K_2-EDTA vacutainer tubes, and subsequently aliquoted and frozen in cryotubes at −80 °C within 1 h of collection in accordance with recommendations from the NIH-CDE Biomarkers Working Group [25]. DNA was extracted from isolated leukocytes using the Wizard® Genomic DNA Purification Kit as described by the manufacturer (Promega, Madison, WI). The ANKK1 C/T SNP (rs1800497) was genotyped utilizing TaqMan®SNP Genotyping Assay as described by the manufacturer (Applied Biosystems, Carlsbad, CA). Patients were categorized by genotype: T/T, C/T, or C/C.

Outcome measures

The primary outcome measure was the California Verbal Learning Test, Second Edition (CVLT-II) Trials 1-5 Standard Score [27], which is one of the "core" TBI CDE outcome measures and was collected in both COBRIT and TRACK-TBI Pilot [28, 29]. The CVLT-II is a verbal learning and memory task in which there are five learning trials, an interference trial, an immediate recall trial, and a post-20 min recall trial. The CVLT-II Trials 1–5 Standard Score (CVLT-TSS) is normed for age and sex, and provides a global index of verbal learning ability [27]. The Wechsler Adult Intelligence Scale processing speed index (WAIS-PSI) was used as a secondary outcome measure [30]. Tertiary outcome measures collected across both studies include the Glasgow Outcome Scale-Extended (GOSE) [31], the Satisfaction with Life Scale (SWLS) [32], the Trail Making Test (TMT) Trail B minus Trail A Score (TMT B-A) [33], and the Brief Symptom Inventory 18 (BSI18) Global Severity Index Score (BSI18 GSI) [34].

Statistical analysis

Primary analysis assessed the impact of the T allele (T/T, C/T, C/C) on the chosen cognitive outcome measures. Group differences in demographic and clinical descriptors across ANKK1 genotypes (T/T, C/T, C/C) were assessed by Pearson's chi-squared test (X^2) for categorical variables and analysis of variance (ANOVA) for continuous variables. Row categories with average cell counts of less than 5 by ANKK1 were combined into a single row category during analysis. Fisher's exact test was used for comparisons with more than 20 % of individual cell counts less than 5. A two-way ANOVA was performed to assess the main effects of ANKK1 dose and study cohort (COBRIT vs. TRACK-TBI) as well as their interaction on 6-month CVLT-TSS. If the interaction was not significant, significant main effects were confirmed with a two-way ANOVA omitting the interaction term, using Tukey's post hoc test with multiple-comparison correction. Fisher's permutation test [35] was performed as a sensitivity analysis to address the unequal distribution of ANKK1 across races. Fifty thousand permutations, within study and race, were used to evaluate the effect of ANKK1. Significance was assessed at $\alpha=0.05$ for all analyses. Fisher's permutation test was performed using Statistical Analysis System (SAS), Version 9.4 (SAS Institute, Cary, NC). All other analyses were performed using Statistical Package for the Social Sciences (SPSS), Version 21 (IBM Corporation, Chicago, IL).

Results

Demographic and clinical descriptors

A total of 492 patients were included in the analysis (COBRIT $N=272$ (55 %), TRACK-TBI Pilot $N=220$ (45 %)). The overall mean age was 40 years old (standard deviation (SD) 16), and subjects were 75 % male (Table 1). The overall race distribution was 78 % Caucasian, 15 % African American/ African, and 2 % or less of each of the other races. Mechanisms of injury were 35 % fall, 24 % motor vehicle accident, 16 % motorcycle/bicycle accident, 13 % assault, 7 % pedestrian struck by vehicle, 3 % struck by/against object, and 2 % other. TBI classification by emergency department (ED) arrival GCS was as follows: 21 % severe (GCS 3–8), 8 % moderate (GCS 9–12), and 71 % mild (GCS 13–15).

Comparison by study demonstrated that there was a lower proportion of African/American-African patients and higher proportions of non-Caucasian, non-African-American/African patients in TRACK-TBI Pilot (Caucasian 75 %, African-American/African 11 %, other 14 %) than in COBRIT (80, 19, and 1 %, respectively, $p<0.001$). Mechanism of injury differed by study ($p<0.001$) with more falls (43 vs. 28 %), fewer motor vehicle accidents (19 vs. 28 %), and fewer motorcycle/bicycle accidents (10 vs. 21 %) observed in TRACK-TBI Pilot than in COBRIT, respectively. COBRIT patients presented with lower GCS (28 % severe, 10 % moderate, 62 % mild) than TRACK-TBI Pilot patients (12 % severe, 5 % moderate, 83 % mild, $p<0.001$). No differences by study were observed in age, gender, or ANKK1 genotype (Table 2).

ANKK1 genotype distribution

ANKK1 genotype distribution was 8 % T/T ($N=40$), 36 % C/T ($N=175$), and 56 % C/C ($N=277$) consistent with the HapMap Phase III average across all races [36]. ANKK1 allelic frequencies ($T=0.26$, $C=0.74$) were found to be at or near Hardy–Weinberg equilibrium ($p=0.263$, Pearson X^2). T

Table 1 Demographic and clinical descriptors by ANKK1 genotype

Baseline variable	All patients	T/T	C/T	C/C	Sig. (p)
Age	N=492	N=40	N=175	N=277	0.861
Mean±SD	40±16	39±13	40±16	41±16	
Gender	N=492	N=40	N=175	N=277	0.404
Male	366	31 (9 %)	124 (34 %)	211 (58 %)	
Female	126	9 (7 %)	51 (41 %)	66 (52 %)	
Race[a]	N=489	N=40	N=174	N=275	<0.001
African-American/African	76	16 (21 %)	32 (42 %)	28 (37 %)	
American Indian/Alaskan	2	2 (100 %)	0 (0 %)	0 (0 %)	
Asian	11	2 (18 %)	5 (45 %)	4 (36 %)	
Caucasian	380	18 (5 %)	128 (34 %)	234 (62 %)	
Hawaiian/Pacific Islander	9	1 (11 %)	4 (44 %)	4 (44 %)	
More than one race	11	1 (9 %)	5 (45 %)	5 (45 %)	
Mechanism of Injury[a]	N=491	N=40	N=174	N=277	0.106
Motor vehicle accident	118	9 (8 %)	49 (42 %)	60 (51 %)	
Motorcycle/bicycle accident	79	5 (6 %)	33 (42 %)	41 (52 %)	
Pedestrian struck by vehicle	33	2 (6 %)	14 (42 %)	17 (52 %)	
Fall	170	11 (6 %)	50 (29 %)	109 (65 %)	
Assault	66	10 (15 %)	17 (26 %)	39 (59 %)	
Struck by/against object	14	1 (7 %)	5 (36 %)	8 (57 %)	
Other	11	2 (18 %)	6 (55 %)	3 (27 %)	
ED arrival GCS	N=489	N=40	N=175	N=274	0.097
Mild (13–15)	348	34 (10 %)	125 (36 %)	189 (54 %)	
Moderate (9–12)	38	2 (5 %)	18 (47 %)	18 (47 %)	
Severe (3–8)	103	4 (4 %)	32 (31 %)	67 (65 %)	

Distribution of demographic and clinical descriptors by ANKK1 genotype. Row percentages are shown for categorical variables (may not equal exactly 100 % due to independent rounding). Statistical significance (p) is assessed using the Pearson chi-squared statistic or Fisher's exact test for categorical variables and ANOVA for continuous variables, by ANKK1 genotype with α=0.05. *ED* Emergency Department, *GCS* Glasgow Coma Scale

[a] Row categories with average cell counts of less than 5 are combined into a single row category during analysis

allele distribution differed across races ($p<0.001$) but conformed to known HapMap Phase III frequencies [36]. Distributions across the two primary race groups in this study were assessed: Caucasians (5 % T/T, 34 % C/T, 61 % C/C) did not differ from the expected CEU HapMap (5 % T/T, 28 % C/T, 66 % C/C ($p=0.291$)), and African American/Africans (21 % T/T, 42 % C/T, 37 % C/C) did not differ from the expected YRI HapMap (16 % T/T, 50 % C/T, 34 % C/C ($p=0.606$)). HapMap comparisons for ANKK1 were not performed for the other races due to small sample sizes of $n\leq10$. No differences in ANKK1 genotype distribution were observed by age, gender, mechanism of injury, or GCS.

Comparison of descriptors between included and excluded patients by study

In both studies, there was a higher proportion of African-American/African patients included in this analysis (COBRIT $N=270$, 80 % Caucasian, 19 % African-American/African, 1 % other; TRACK-TBI Pilot $N=220$, 75 % Caucasian, 11 % African-American/African, 14 % other) compared to patients not included (COBRIT $N=938$, 83 % Caucasian, 13 % African-American/African, 4 % other, $p=0.033$; TRACK-TBI $N=348$, 85 % Caucasian, 6 % African-American, 9 % other, $p=0.043$). The included COBRIT patients had less severe injuries by GCS ($N=271$, 28 % severe, 11 % moderate, 62 % mild) compared to those not included ($N=936$, 39 % severe, 10 % moderate, 51 % mild, $p=0.004$). The included TRACK-TBI Pilot patients were younger ($N=220$, mean 41, SD 16) compared to those not included ($N=352$, mean 46, SD 19). No differences in other baseline descriptors or ANKK1 genotype distribution were observed between included and excluded adult patients within each study (Online Resource 1 and 2).

Comparison of descriptors between COBRIT treatment and control arms

The COBRIT patients included in this analysis ($N=272$) distributed evenly across citicoline ($N=137$ (50 %)) and placebo arms ($N=135$ (50 %)). No differences in any demographic and clinical descriptors were observed by treatment arm (Online Resource 3).

Table 2 Demographic and clinical descriptors by study

Baseline variable	COBRIT	TRACK-TBI Pilot	Sig. (p)
Age	N=272	N=220	0.453
Mean±SD	40±15	41±16	
Gender	N=272	N=220	0.072
Male	211 (78 %)	155 (70 %)	
Female	61 (22 %)	65 (30 %)	
Race[a]	N=270	N=219	<0.001
African-American/African	51 (19 %)	25 (11 %)	
American Indian/Alaskan	0 (0 %)	2 (1 %)	
Asian	2 (1 %)	9 (4 %)	
Caucasian	216 (80 %)	164 (75 %)	
Hawaiian/Pacific Islander	0 (0 %)	9 (4 %)	
More than one race	1 (0 %)	10 (5 %)	
Mechanism of injury	N=272	N=219	<0.001
Motor vehicle accident	77 (28 %)	41 (19 %)	
Motorcycle/bicycle accident	58 (21 %)	21 (10 %)	
Pedestrian struck by vehicle	13 (5 %)	20 (9 %)	
Fall	76 (28 %)	94 (43 %)	
Assault	33 (12 %)	33 (15 %)	
Struck by/against object	8 (3 %)	6 (3 %)	
Other	7 (3 %)	4 (2 %)	
ED arrival GCS	N=271	N=218	<0.001
Mild (13–15)	168 (62 %)	180 (83 %)	
Moderate (9–12)	27 (10 %)	11 (5 %)	
Severe (3–8)	76 (28 %)	27 (12 %)	
ANKK1 genotype	N=272	N=220	0.193
T/T	17 (6 %)	23 (11 %)	
C/T	102 (38 %)	73 (33 %)	
C/C	153 (56 %)	124 (56 %)	

Distribution of demographic and clinical descriptors by study. Column percentages are shown for categorical variables (may not equal exactly 100 % due to independent rounding). Statistical significance (p) is assessed using the Pearson chi-squared statistic or Fisher's Exact Test for categorical variables, and ANOVA for continuous variables, by ANKK1 genotype with α=0.05. *ED* Emergency Department, *GCS* Glasgow Coma Scale

[a] Row categories with average cell counts of less than 5 are combined into a single row category during analysis

Relationship of ANKK1 to CVLT-TSS

Our analyses were designed to address potential confounding created by pooling COBRIT and TRACK-TBI Pilot data for the effects of the following: (1) the particular study and (2) interaction between ANKK1 and the particular study on CVLT-TSS. First, we performed a two-way ANOVA with CVLT-TSS as the dependent variable to assess the main effects of ANKK1 and study, plus the interaction term ANKK1 X study. Table 3 shows that ANKK1 had a statistically significant association at α=0.05 with CVLT-TSS (F(2, 486)=4.964, p=0.007), while particular study and ANKK1 X

Table 3 Association of ANKK1 genotype and study with 6-month CVLT-TSS

Source	Type III sum of squares	df	Mean square	F	p value
Corrected model	1773.6	5	354.7	1.993	0.078
ANKK1	1767.4	2	883.7	4.964	0.007
Study	54.2	1	54.2	0.304	0.581
ANKK1 X study	35.0	2	17.5	0.098	0.906
Error	86517.8	486	178.0		

Two-way ANOVA to assess the main effects of ANKK1 genotype (abbreviated as ANKK1) and Study (COBRIT or TRACK-TBI Pilot) plus the interaction term (ANKK1 X Study) on six-month CVLT-TSS as the dependent variable in the model. Significant assessed at α = 0.05

CVLT-TSS California Verbal Learning Test, Second Edition Trials 1-5 Standard Score, *df* degrees of freedom

study did not. We then re-ran the model, omitting the interaction term, to confirm the significant association between ANKK1 and CVLT-TSS (F(2,486)=4.893, p=0.008), and not between particular study and CVLT-TSS (F(1,486)= 0.117, p=0.732). We performed Tukey's post-hoc test for ANKK1 in the same model to assess for differences in CVLT-TSS across the three ANKK1 genotypes. Figure 1
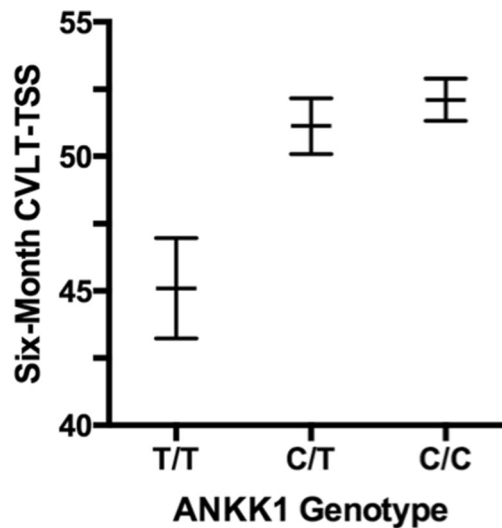
ANKK1 Genotype (I vs. J)	Mean CVLT-TSS (SE) (I vs. J)	MD (95% CI) (I minus J)	p-value
T/T vs. C/T	45.1 (1.9) vs. 51.1 (1.0)	-6.0 (-11.5, -0.5)	0.027
T/T vs. C/C	45.1 (1.9) vs. 52.1 (0.8)	-7.0 (-12.3, -1.7)	0.006

Fig. 1 Comparison of 6-month CVLT-TSS means across ANKK1 genotypes. Graph shows 6-month CVLT-TSS mean±SE by ANKK1 genotype. Tukey's post hoc test was used to assess mean differences (MD) in CVLT-TSS between genotypes. Only significant MDs at α=0.05 are shown in the table. Mean difference is calculated by the mean CVLT-TSS of the first genotype (I) minus that of the second genotype (J). *CVLT-TSS* California Verbal Learning Test, Second Edition Trials 1–5 Standard Score, *SE* standard error, *CI* confidence interval

shows the CVLT-TSS means by ANKK1, and that mean CVLT-TSS of T/T patients differed significantly from that of C/T and C/C patients, with a mean decrease of 6.0 points against C/T and 7.0 points against C/C.

Based on our initial descriptive statistics (Table 1), there were subpopulation differences in the distribution of ANKK1 genotypes across races. As a sensitivity analysis, we ran Fisher's permutation test as a distribution-free alternative to the parametric model [35]. The association between ANKK1 and six-month CVLT-TSS remained significant ($p=0.026$) when controlling for race and particular study.

Exploratory analysis of ANKK1 on other outcome measures

To explore the common six-month outcome measures in our pooled multicenter dataset, we assessed the association between ANKK1 genotype on a non-verbal cognitive test, the WAIS-PSI, as well as with four other measures: GOSE, SWLS, TMT B-A, BSI18 GSI. We performed identical analyses as above to assess the main effect of ANKK1 genotype and particular study, plus the interaction factor ANKK1 X study, using two-way ANOVA with each outcome measure as the dependent variable. There was a significant association at $\alpha=0.05$ between ANKK1 and WAIS-PSI ($F(2,486)=3.225$, $p=0.041$), and particular study and WAIS-PSI ($F(1,486)=7.01$, $p=0.008$), with no effect of ANKK1 X study. No significant pairwise differences at $\alpha=0.05$ were observed in WAIS-PSI means across ANKK1 (T/T: 94.1, SE 2.5; C/T: 95.9, SE 1.3; C/C: 98.8; SE 0.9) on Tukey's post-hoc test. Mean WAIS-PSI scores in COBRIT were lower than in TRACK-TBI Pilot (COBRIT: 95.9, SE 1.0; TRACK-TBI Pilot 99.3, SE 1.1, $p=0.02$). There was no significant association between ANKK1, study or ANKK1 X study with GOSE, SWLS, or TMT B-A. There was a marginal association at $\alpha=0.05$ between ANKK1 and BSI18 GSI ($F(2,486)=3.0$, $p=0.052$), with no effect of particular study or ANKK1 X study. On Tukey's post hoc test, BSI18 GSI means (T/T 60.1, SE 2.1; C/T 54.7, SE 0.9; C/C 56.3, SE 0.7) differed significantly at $\alpha=0.05$ between T/T and C/T only (95 % CI 0.3 to 10.4, $p=0.036$).

Discussion

Over the past decade, genetic association studies have contributed to our understanding of the molecular mechanisms of multiple common human diseases, including Alzheimer disease, heart disease, and diabetes, among others [37–42]. In each case, molecular mechanisms suspected to be involved in disease pathogenesis based on preclinical or pathologic studies were confirmed by human genetics. In addition, human genetic association studies have uncovered new molecular pathways previously unsuspected to play a role in disease

pathogenesis [43–47]. The overwhelming majority of these genetic discoveries, however, have applied to disease risk [48–52]. TBI presents special challenges for genetic association studies [53]. First, there is a prominent and stochastic environmental factor: the traumatic injury. Second, premorbid personality and developmental factors play a clear role in recovery from injury. Thus, in order to identify molecular pathways in resilience to or recovery from TBI, large sample sizes and collection of comprehensive data, which allow for consideration of premorbid factors and assessment of injury severity, are essential [54, 55]. The use of CDEs is fundamental to the success of these efforts and the NIH-NINDS TBI CDEs were designed to address this need [21]. Investigators of COBRIT and TRACK-TBI Pilot were among the leaders in this effort, and the present study was feasible because of the high degree of overlap between the assessment tools and outcome measures utilized in the two studies.

Our robust sample permitted confirmation of the hypothesis concerning the effects of the T allele on cognitive outcome. Indeed, we found an association between ANKK1 and poorer performance on 6-month CVLT-TSS specifically tied to the T/T genotype. The C/T group alone did not show any differences from the C/C group on CVLT-TSS. Although this does not align perfectly with previous findings in TBI, where T-allele carriers showed worse performance on an episodic memory task of the CVLT, our overall result remains more confirmatory than divergent. McAllister et al. reported only one T/T individual in a sample size of 141, which could not enable a T-dose-dependent analysis. The distribution ANKK1 genotypes in our analysis approaches that of the general population according to HapMap Phase III and therefore allows us more statistical power to investigate the differential relationships between genotype and cognition. Secondly, it may be that differential genotypic associations with specific symptoms are more easily identified on specialized verbal memory trials such as the CVLT recognition task while the deleterious effect of a double dose of T allele manifest on the CVLT-TSS, a more highly generalizable and normative global index of verbal learning ability.

Our study reinforces the benefits of pooling multicenter trials into a unified data commons. There were no differential study effects by COBRIT and TRACK-TBI Pilot, nor were there ANKK1 X study interactions, on six-month verbal learning. This validates data sharing as a mechanism to raise statistical power for hypothesis testing and increases our confidence in the associations of ANKK1 T/T with verbal learning across a large, heterogeneous TBI population.

As well, merging COBRIT and TRACK-TBI Pilot data effectively captures patients across the entire TBI spectrum. As COBRIT excluded patients with GCS 13–15 presenting with negative head CTs, it targeted patients with more moderate and severe TBI whereas TRACK-TBI Pilot enrolled patients with similar TBI incidence as reported in literature and the

population, which is predominantly mild [56, 57]. Indeed, COBRIT patients in the current analysis presented with more severe TBI compared to TRACK-TBI Pilot, and this difference may account for the observed differences by study in some of our analyses of secondary outcomes. For example, the study effect on WAIS-PSI scores reached significance. It is also interesting to note the marginal signal of ANKK1 T/T with the BSI18 GSI, which corroborates the range of studies interrogating ANKK1 in the context of neuropsychiatric disorders.

Our study has clarified several key areas identified by McAllister et al. as areas of further investigation concerning the relationship of ANKK1 with TBI outcome [11, 12]. The authors questioned whether their results would hold in a larger, more diverse racial and ethnic population, with varying injury severity and in outcomes at a longer post-injury interval. By utilizing two multicenter studies (COBRIT: eight centers, TRACK-Pilot: three centers, one center participated in both studies), the sample size was expanded to encompass a total of 10 Level I trauma centers across the USA. This heterogeneous population covers the full severity spectrum from concussion to coma, which previous studies did not have an opportunity to evaluate in the context of ANKK1. Regarding outcomes, McAllister et al. were only able to access CVLT at 1-month post-injury and expressed concern about generalizability at later timepoints. With a larger multicenter sample and long-term follow-up (the 6-month clinical standard), the present study is more resilient to local demographic and practice effects providing a strong replication test of McAllister et al.'s results.

Limitations

Although we have improved upon the breadth and generalizability of previous studies, we recognize several limitations in the current analysis. First, we could not fully account for the impact of TBI pathology and lesion types on recovery, the lack of pre-injury psychometric tests, other genetic predispositions, and non-TBI control groups. As our primary analysis was confirmatory in nature, we pursued similar inclusion criteria as McAllister et al. for general TBI and did not explore the structure-function implications of ANKK1 with intracranial lesion types or baseline mental health variables. Given the heterogeneity of TBI, subjects may never be perfectly matched by type, location, and extent of injury. Despite this fact, convincing evidence of genetic association can be clarified by sufficiently large sample sizes. The ability to comment on causative or confounding relationships between ANKK1 and pre- or post-injury risk factors is beyond the scope of the current analysis. As T/T has been associated with propensity for addiction and poor coping strategies [8, 14–17, 58, 59], the acquisition and analysis of detailed pre-injury addictive behavior, post-acute treatment, and recovery variables are relevant next steps in delineating the contribution of ANKK1 to both TBI risk and outcome variability. We are also constrained by

the lack of genome-wide data, which makes it difficult to fully control for population stratification, as evidenced by the observed differences for patients who met the inclusion criteria for this analysis compared their excluded counterparts in COBRIT and TRACK-TBI Pilot. The proportion of T/T within our sample is still rather small, limiting our ability to assess whether there is a differential influence of ANKK1 genotypes on other domains of outcome, or in different races. The robustness of the association between ANKK1 and a given outcome domain such as working memory or processing speed, which encompasses multiple individual outcome measures, can be interrogated using multivariate integration and correlated with specific injuries in the dorsolateral prefrontal cortex—where working memory processes are known to be confined [60–62]. Work of this type is ongoing in the TRACK-TBI consortium.

In analyzing patients with full outcomes, there is an inherent risk of selecting for patients able to return for follow-up. For example, in our study, the COBRIT patients with genotyping and complete six-month outcomes presented with less severe injuries than those who had incomplete outcomes. This may be attributable in part to better cognition and functional ability to return for follow-up. As observed in TRACK-TBI Pilot, patients of younger age may be more mobile and/or available to return for full outcomes assessment. In some ways, the selection bias relates to the primary goal of this analysis, which was to assess the association of ANKK1 with outcome measures common to both studies and hence contingent on patients with valid scores. It is difficult to capture reasons for incomplete outcomes in patients who are lost to follow-up, as in many cases contact is never made.

The molecular mechanism and active location of ANKK1 remains a topic of ongoing study, with further experiments needed in cellular and animal models, as well as human trials. There is a need to examine gene-gene interaction with other loci of susceptibility for prognostic phenotyping within the dopaminergic system to elucidate an ANKK1 molecular pathway in local CNS physiology, contingent on detailed structure-function analysis from the comprehensive mapping of the human connectome [63]. Alternatives to the limitations of conventional imaging modalities such as CT are being explored with TRACK-TBI Pilot data. Early results indicate that prediction models including contusion on 3T MRI and axonal injury by diffusion tensor imaging (DTI) surpass other predictors for global outcome prediction in a subset of patients after mild TBI [64]. Advanced diffusion imaging modalities targeting the dorsal prefrontal cortex have been reported for healthy and diseased states [65–69]. Increased precision in characterizing regional pathophysiology will enable more objective control of injury type and severity in order to distill the specific mechanism by which ANKK1 modulates working memory, as a subset of the disparate patterns of cognitive impairment observed in the current TBI classification system of mild, moderate, and severe. In a broader sense, further

development of classification approaches based on quantitative morphometry [70], in conjunction with appropriate computational methods [71] and data integration processes [72], will aid in deconstructing the contribution of genetic modulation to multidimensional domains of outcome after TBI.

Greater sample size and more extensive genotyping will overcome our current limitations to allow for stratification across known genetic profiles and TBI severities, as well as raise statistical power to levels appropriate for phase III clinical trials. We successfully pooled COBRIT and TRACK-TBI Pilot data through outcome measures common to both studies, but we were still constrained in our scope of data pooling. Clearer evaluations of the effects of risk factors and predictors of TBI outcome, including ANKK1 and other SNPs, await the expanded initiatives of current multicenter studies such as the Transforming Research and Clinical Knowledge in TBI study (TRACK-TBI) [73] and the Collaborative European NeuroTrauma Effectiveness Research in TBI study (CENTER-TBI) [74], which will enroll 3000 and 5000 patients with controls, respectively, over the next five years, using the expanded Version 2 of the NIH-NINDS TBI CDEs [21, 75]. Adopting an international approach [76] to this standardized set of variables with wide scope, utility, and applicability will allow us to converge and leverage research efforts to achieve the sample sizes we truly need for delineating the effects of the ANKK1 polymorphism in TBI.

Conclusions

In the largest prospective multicenter study to date examining the incidence of the rs1800497 SNP in TBI, enabled by data pooling of shared common variables, we report that the ANKK1 T/T genotype associates with poorer verbal learning performance on CVLT-TSS at six months post-injury across the spectrum of TBI severity. With the augmented statistical power of this analysis, successful replication of the association between ANKK1 and cognition reinforces the potential implication of a DRD2-dependent biological mechanism underlying cognitive performance after TBI.

Compliance with Ethical Standards

Disclosure of Potential Conflicts of Interest   The authors declare that they have no conflicts of interest.

Research Involving Human Participants   All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent   Informed consent was obtained from all individual participants included in the study.

Appendix

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