



A genome-wide association study of outcome from traumatic brain injury

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Summary

Background Factors such as age, pre-injury health, and injury severity, account for less than 35% of outcome variability in traumatic brain injury (TBI). While some residual outcome variability may be attributable to genetic factors, published candidate gene association studies have often been underpowered and subject to publication bias.

Methods We performed the first genome- and transcriptome-wide association studies (GWAS, TWAS) of genetic effects on outcome in TBI. The study population consisted of 5268 patients from prospective European and US studies, who attended hospital within 24 h of TBI, and satisfied local protocols for computed tomography.

Findings The estimated heritability of TBI outcome was 0.26. GWAS revealed no genetic variants with genome-wide significance ($p < 5 \times 10^{-8}$), but identified 83 variants in 13 independent loci which met a lower pre-specified sub-genomic statistical threshold ($p < 10^{-5}$). Similarly, none of the genes tested in TWAS met tissue-wide significance. An exploratory analysis of 75 published candidate variants associated with 28 genes revealed one replicable variant (rs1800450 in the *MBL2* gene) which retained significance after correction for multiple comparison ($p = 5.24 \times 10^{-4}$).

Interpretation While multiple novel loci reached less stringent thresholds, none achieved genome-wide significance. The overall heritability estimate, however, is consistent with the hypothesis that common genetic variation

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Abbreviations: TBI, Traumatic brain injury; GWAS, Genome wide association study; TWAS, Tissue wide association study; SNV, Single nucleotide variant; GCS, Glasgow coma scale; GOSE, Glasgow outcome scale - extended; MRI, Magnetic resonance imaging

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substantially contributes to inter-individual variability in TBI outcome. The meta-analytic approach to the GWAS and the availability of summary data allows for a continuous extension with additional cohorts as data becomes available.

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Keywords: Traumatic brain injury; Genome-Wide association study; Outcome; Recovery; Consortia

Research in context

Evidence before this study

Even the best multivariable prognostic models account for ~35% of inter-individual variability in outcome from moderate-severe traumatic brain injury (TBI), and perform even worse in mild TBI. Correction for between-centre differences, and more precise characterization of injury severity (e.g., using MRI and blood-based biomarkers) still leaves over 50% of outcome variation unexplained. This suggests that host-specific factors, not just injury severity, play important roles in outcome. Highly penetrant rare genetic variants can cause life-threatening brain swelling in response to trivial head injury, and several candidate gene studies have offered preliminary knowledge on the role of common variants in TBI outcome. Recent systematic reviews concluded that existing publications regarding the impact of genetic variation on TBI outcome were limited by small sample size, publication bias, and other shortcomings of study design. In particular, there has been no rigorously conducted, large, unbiased genome-wide association study (GWAS) examining the impact of common genetic variation on TBI outcome.

Added value of this study

We have conducted the first GWAS of TBI outcome, using a sample size at least four times larger than any previous study. The estimated heritability of the impact of genetic variation on outcome was 26%, which is within the range of common neurological diseases that have recognised genetic associations. While none of the associations in our analysis reached genome-wide significance, several achieved statistical thresholds that merit further investigation. A supplementary transcriptome-wide association study (TWAS) of genetically regulated gene expression did not identify genes achieving genome-wide significance, but identified several biologically plausible associations.

Implications of all the available evidence

The heritability estimation confirms the hypothesis that host genetic variation does indeed play a role in TBI outcome. Nonetheless, even a sample size of ~5000 appears to be underpowered to identify specific common-variant genetic effects in such a complex phenotype. The failure for replication of previously published

candidate single nucleotide variants (SNVs) or genes to show a significant effect underlines the need for caution in making inferences from candidate gene studies, although they continue to have a clear role where there is a strong biological rationale and a more quantifiable outcome. The heritability estimate, along with identification of associations through GWAS and TWAS analyses at lower thresholds of significance, make a case for extending the current meta-analysis with additional follow-up studies to increase overall sample size and thus power. Furthermore, the failure to demonstrate a clear association with GOSE might indicate the need to additionally explore more precise outcomes directly related to underlying biology.

Introduction

Traumatic brain injury (TBI) is a leading cause of mortality and disability.^{1–3} While severity of initial injury varies from mild to moderate to severe <35% of the inter-individual variability in outcomes is explained by injury severity, age, or pre-injury health.^{4,5} A fraction of residual variability in outcome is attributable to practice variation.⁶ The vast majority remains unexplained. Factors specific to the individual TBI victim likely play a substantial role.

Genetic variation can have a potent effect on individual response to TBI.^{7,8} Mutations in *CACNA1A* or *Na⁺/K⁺ ATPase*, for example, can cause life-threatening brain swelling in response to trivial head injuries.^{9,10} The present study is the first step toward identifying common genetic variants that modulate a person's response to TBI. Such variants offer the promise of yielding novel targets for desperately needed therapies that could dampen the “dose” of neurotrauma, or improve the trajectory of recovery and ultimate functional outcome.

Prior studies of TBI have been limited to small, underpowered candidate gene association studies with inconclusive and sometimes contradictory results.^{7,8} Major challenges in undertaking an appropriately powered GWAS in TBI include assembling sample sizes with adequate statistical power, ensuring that phenotyping is harmonised across cohorts, and that outcome assessment is uniform.

The International Traumatic Brain Injury Research (InTBIR, <https://intbir.nih.gov>) initiative has generated well-characterized study cohorts with detailed clinical, neuroimaging, and outcome assessment, and blood banked for genetic analysis. The two largest InTBIR studies: CENTER-TBI¹¹ (Collaborative European Neuro-Trauma Effectiveness Research, <https://www.center-tbi.eu>) and TRACK-TBI¹² (Transforming Research and Clinical Knowledge in TBI, <https://tracktbi.ucsf.edu>) utilized the NIH/NINDS Common Data Elements (<https://www.commondataelements.ninds.nih.gov>), to ensure harmonization. Smaller cohorts from Cambridge (UK), Turku (Finland), and Mass General Brigham (MGB; Boston, USA) which banked DNA have also collected such standardized phenotypic data.

These groups formed the Genetic Associations in Neurotrauma (GAIN) Consortium to perform the first GWAS and transcriptome-wide association study (TWAS) in TBI. In addition, we report a targeted analysis of previously reported TBI candidate gene variants.

Methods

Data are reported in compliance with STREGA¹³ guidelines (Supplementary Materials). Individuals included were recruited between 2000 and 2018 at 78 centres in Europe (CENTER-TBI, Cambridge, and Turku) and the US (TRACK-TBI and MGB) (Supplementary Methods). All patients presented to hospital with TBI within 24 h of injury, and underwent head CT imaging. Outcomes were measured using the extended Glasgow Outcome Scale (GOSE), ranging from 1 (dead) to 8 (upper good recovery), measured 6 months post-TBI. For individuals in CENTER-TBI and TRACK-TBI in whom GOSE at 6 months was missing, but GOSE was measured at another time point within one year of the injury, missing 6-month GOSE values (499/2455 in the core CENTER-TBI and 274/1672 in the TRACK-TBI cohort) were imputed using a Markov multi-state model exploiting available longitudinal GOSE measurements. Used multi-stage approach relies on Markov assumptions and allows to model the probability of transitions between GOSE states and outperforms alternative panel imputation methods as discussed in detail by Kunzmann et al.¹⁴ Where no GOSE values were available at any time point, patients were excluded from the analysis. TBI severity was specified using the Glasgow Coma Score (GCS), with TBI classified as mild (GCS 13-15), moderate (GCS 9-12), or severe (GCS 3-8).

To account for the effect of injury severity on outcome, we used sliding dichotomization¹⁵ to categorize outcome as favourable or unfavourable. A GOSE ≤ 4 was used to define an unfavourable outcome for patients with either moderate (GCS 9-12) or severe (GCS 3-8) TBI, while the unfavourable group was extended to patients with GOSE ≤ 7 if they had mild

(GCS 13-15) TBI (Supplementary Methods, Supplementary Fig. 1).

Statistics

Genotyping was completed at FIMM Technology Center for CENTER-TBI, Cambridge, Turku patients and the Broad Institute for TRACK-TBI, using the Illumina Global Screening Array (GSA-24v2-o + Multi-Disease). The MGB cohort were genotyped using Illumina's Multi-Ethnic Global array (MEGA) and the pre-releases forms, including MEGA and MEGA-Ex arrays at Illumina at the MGB Translational Genomics Core. A unified quality control procedure was applied for each study cohort and the array-based genotypes were imputed using the Haplotype Reference Consortium¹⁶ panel. Details regarding genotyping and imputation are in the Supplementary Methods.

TBI patients' ancestry were determined by self-reports and confirmed through principal components (PCs) calculated based on the genotypes of the study population combined with the genotypes of the 1000 Genomes¹⁷ reference data (Supplementary Methods). The final data set contained 4710 individuals of European ancestry. TRACK-TBI patients clustered to the 1000 Genomes Africans ($n = 245$) and Admixed Americans ($n = 313$) ethnic groups were included in the trans-ethnic GWAS meta-analysis, allowing us to constitute a multi-ethnic cohort of 5268 individuals. Target sample size was not defined *a priori*, but was the largest combined cohort of well-phenotyped patients with outcomes, and DNA that satisfied quality control requirements.

Genome-wide association analysis and meta-analysis

Genome-wide single-marker scans were performed using a penalized likelihood-based Firth logistic regression, and implemented in PLINK¹⁸ v2.0. Using favourable outcome as reference, models were fitted on the basis of imputed allelic dosages. Age, sex, major extracranial injury (MEI) (Supplementary Methods), and pupillary reactivity were included as covariates, also the first 10 PCs to reduce the confounding effect introduced by population structure. Study cohort (CENTER-TBI, Cambridge, Turku) was an additional covariate in the CENTER-TBI GWAS.

Fixed-effects meta-analysis of the three European ancestry GWAS was performed using METAL.¹⁹ For trans-ethnic meta-analysis, summary statistics of five GWASs in patients of European, African and Admixed Americans were aggregated via MR-MEGA²⁰ v.o.1.6. To examine associations with isolated TBI, we undertook a secondary analysis confined to patients without the confounding effects of MEI.

For the genome-wide meta-analyses, significance was set at $p < 5 \times 10^{-8}$ and a second, less stringent,

sub-genome-wide significance level of interest at $p < 10^{-5}$. Analysis details are provided in the Supplementary Methods.

Candidate gene analyses

We performed further *in silico* analysis of single nucleotide variants (SNVs) in 28 genes identified through systematic review of published candidate gene studies (Supplementary Table 5). The majority of these studies had been conducted with small sample sizes ($n < 100$). We examined associations in candidate genes if SNVs could be imputed with minor allele frequency (MAF) $> 1\%$ in all three study cohorts. We applied a Bonferroni corrected significance of 6.67×10^{-4} ($0.05 / 75$ SNVs tested).

For *APOE*,⁸ individuals were assessed as ϵ_4 carriers and non-carriers (rs429358 and rs7412). We assessed the impact of ϵ_4 on outcome (both sliding dichotomy approach and the range of ordinal GOSE) across the entire cohort and by TBI severity subsets. Pearson's chi-squared test was used to identify differences in outcome distribution between *APOE* ϵ_4 carriage groups.

Transcriptome-wide association study

Genetically regulated gene expression (GREx) was imputed using a regression model fitted on a separate gene expression database, consistent with standard approaches.^{21–23} Elastic net models provided by PrediXcan^{24,25} for all available GTEx brain tissues and whole blood were used (Supplementary Table 2). Imputed SNVs were pre-filtered by imputation quality (INFO > 0.8 for CENTER-TBI, $R^2 > 0.8$ for TRACK-TBI and MGB) and MAF $> 1\%$. The resulting number of distinct genes per tissue for which genetically regulated gene expression could be imputed is given in the Supplementary Table 2. The overall number of unique genes and transcripts for which genetically regulated gene expression is available in at least one tissue is 15104 (39.7%). Tissue-specific Bonferroni-corrected significance level of 0.05 based on the number of imputed genes per each tissue was used.

For TWAS, we used the same sliding dichotomy model for outcome with the same set of covariates as in the GWAS, but PCA components were replaced with the top five PCs of the respective gene expression data. The same set of 28 candidate genes separately assessed were also studied in TWAS. We were unable to examine seven genes (*ABCB1*, *AQP4*, *IL1A*, *IL1B*, *IL6*, *MBL2*, and *OPRM1*) because they were not available in any of the tissues of interest with PrediXcan.

Ethics statement

For the main CENTER-TBI cohort ethical approval was obtained separately within each country. As an example, permission in the UK was obtained from the National

Research Ethics Committee East of England – Norfolk (12/EE/0395) - other national ethical approvals are detailed on the CENTER-TBI website (<https://www.center-tbi.eu/project/ethical-approval>). The historical Cambridge cohort was recruited with ethical permission from Cambridgeshire Research Ethics Committee (REC 97/290), the Turku patients based on approval from the review board of the Hospital District of Southwest Finland (decision 68/180/2011). Ethical approval for the TRACK-TBI study was provided by the San Francisco General Hospital Panel Institutional Review Board (IRB #: 12-09465; Reference #: 313687). The MGB study was performed in compliance with the privacy and data protection regulations as defined by the Mass General Brigham Biobank (IRB #: 2017P002397). Further details are available in the Supplementary Methods.

Role of funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, and writing of the report.

Results

Subjects were predominantly men (53.75% across cohorts), with a mean age ranging from 34 to 61 years. The majority (59.91% across cohorts) of cases had mild TBI (GCS 13–15). Differences in TBI severity were associated with differences in GOSE in each cohort: mortality was between 2% and 10%; combined death or severe disability between 9% and 25%; and complete recovery between 24% and 41% (Table 1, Supplementary Table 1).

Genome-wide association meta-analyses

Primary analysis. 4710 individuals of European ancestry were included from CENTER-TBI ($n = 3187$), TRACK-TBI ($n = 1114$) and MGB ($n = 409$). Heritability estimate (liability scale) of TBI outcome was $h_g^2 = 0.26$. Following post-imputation data quality control (INFO > 0.4 or $R^2 > 0.4$, MAF $> 1\%$), we assessed associations of 7246366 imputed autosomal variants common to all three datasets in 2509 unfavourable and 2201 favourable TBI outcome patients. Individual contributory study-level Manhattan plots are presented in the Supplementary Fig. 2. The results were combined using a fixed-effect meta-analysis (the genomic inflation factor, $\lambda = 0.983$). No genetic variant reached genome-wide significance ($p < 5 \times 10^{-8}$) (Figure 1), but we detected 83 variants in 13 loci associated with TBI outcome, which met a less stringent, pre-specified sub-genome-wide threshold of interest ($p < 10^{-5}$) (Table 2). All but one (rs1047208 in *MYO1D* gene) of these signals were

Ancestry	CENTER-TBI*		TRACK-TBI		MGB
	European	European	African	Ad Mixed American	European
Patients (n)	3187	1114	245	313	409
Age (mean [sd; range])	47 (19; 16-80)	44 (18; 18-90)	39 (15; 18-76)	34 (13; 18-72)	61 (17.5; 15-95)
Sex: female	952 (30%)	343 (31%)	83 (34%)	78 (25%)	191 (47%)
Major Extracranial Injury	1173 (37%)	208 (19%)	50 (20%)	55 (18%)	107 (26%)
Pupillary reactivity:					
Both reacting	2782 (87%)	1042 (94%)	233 (95%)	294 (94%)	398 (97%)
One reacting	186 (6%)	15 (1%)	3 (1%)	3 (1%)	3 (1%)
Both unreactive	219 (7%)	57 (5%)	9 (4%)	16 (5%)	8 (2%)
Glasgow Coma Scale:					
13-15 (mild TBI)	1866 (59%)	884 (79%)	201 (82%)	235 (75%)	371 (90.7%)
9-12 (moderate TBI)	333 (10%)	46 (4%)	9 (4%)	30 (10%)	19 (4.65%)
3-8 (severe TBI)	988 (31%)	184 (17%)	35 (14%)	48 (15%)	19 (4.65%)
GOSE at 6 months:					
1	327 (10.3%)	70 (6.3%)	6 (2.4%)	9 (2.9%)	20 (4.9%)
2 or 3	332 (10.4%)	57 (5.1%)	19 (7.8%)	32 (10.2%)	6 (1.5%)
4	169 (5.3%)	28 (2.5%)	6 (2.4%)	3 (1.0%)	11 (2.7%)
5	352 (11.0%)	120 (10.8%)	34 (13.9%)	39 (12.5%)	27 (6.6%)
6	396 (12.4%)	211 (18.9%)	69 (28.2%)	74 (23.6%)	43 (10.5%)
7	567 (17.8%)	278 (25.0%)	47 (19.2%)	82 (26.2%)	135 (33.0%)
8	1044 (32.8%)	350 (31.4%)	64 (26.1%)	74 (23.6%)	167 (40.8%)
TBI outcome:					
Unfavorable**	1613 (51%)	674 (61%)	162 (66%)	206 (66%)	222 (54%)
Favorable	1574 (49%)	440 (39%)	83 (34%)	107 (34%)	187 (46%)

Table 1: Characteristics of the TBI patients.
GOSE: Glasgow Outcome Scale (ranging from Death [GOSE = 1] to Upper Good Recovery [GOSE = 8]); sd: standard deviation.
* Cohorts recruited in Europe (CENTER-TBI, Cambridge and Turku).
** Defined using sliding dichotomy: GOSE ≤ 7 for patients with mild TBI (GCS 13-15) and GOSE ≤ 4 for patients with moderate (GCS 9-12) and severe (GCS ≤ 8) TBI.

driven by the largest study cohort (CENTER-TBI) (Supplementary Table 3).

Among those associations achieving sub-genome-wide significance ($p < 10^{-5}$), rs2390015 on chromosome 1, achieved the lowest p-value ($p = 1.34 \times 10^{-6}$) (Table 2). Located in an intronic region of *COL24A1* gene, this variant is predicted to act as an expression quantitative trait locus (eQTL) by GTEx database, potentially altering *COL24A1* expression. Among the other top hits, rs6543009, located in chromosome 2, in an intronic region of the *TBC1D8* gene, was predicted acting as eQTL and splicing quantitative trait locus (sQTL), possibly modifying *TBC1D8* and *RPL31* gene expression and splicing in different tissues, with potential neurological associations.^{26,27} A third eQTL variant, rs1047208 on chromosome 17, was located in the 3' UTR region of *MYO1D*.²⁸ GWAS Catalog and LDlink queries highlighted that none of the findings were previously identified as significant by other association studies, nor being in strong linkage disequilibrium with previously published GWAS hits.

A secondary analysis in a subgroup of patients with no major extracranial injury ($n = 3223$) showed no

genomic variants with genome-wide significance (Supplementary Table 4, Supplementary Fig. 3).

Candidate gene analysis. We explored 28 candidate genes previously reported with GOSE. After Bonferroni correction, the rs1800450 exonic polymorphism in *MBL2* demonstrated statistically significant association with TBI outcome ($p = 5.24 \times 10^{-4}$), followed by three nominally significant ($p < 0.05$, uncorrected) associations (rs1800629 in *TNF*; rs5030737 and rs7096206 in *MBL2*) (Supplementary Table 5). None of the other previously published variants showed significant associations with TBI outcome.

APOE ε4. *APOE* ε4 showed no association with TBI outcome in the overall cohort ($p = 0.70$) nor TBI severity subsets (p-values for mild, moderate and severe TBI patients were 0.90, 0.18 and 0.78, respectively) using sliding dichotomisation. Exploring the entire ordinal range of GOSE, ε4 non-carriers with mild TBI achieved a significant shift in GOSE compared to carriers with mild TBI ($p = 0.035$) (Supplementary Fig. 4). We did

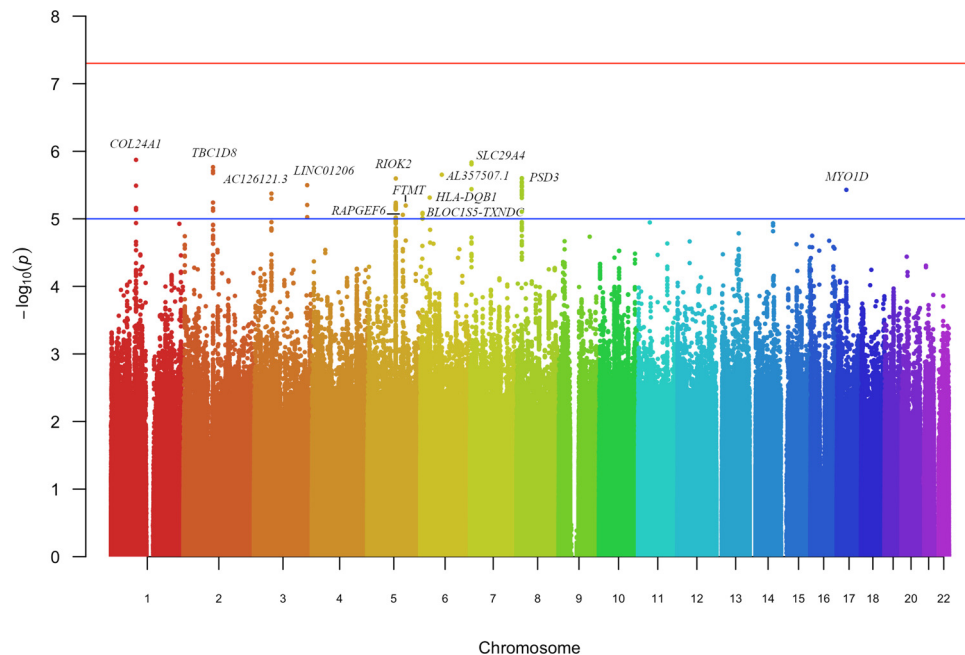


Figure 1. Manhattan plot of European ancestry meta-analysis for TBI outcome ($n = 4710$). The red line indicates the genome-wide significance level threshold to account for multiple testing ($p < 5 \times 10^{-8}$) and the blue line indicates a sub-genome-wide significance level of $p < 10^{-5}$.

not observe such differences for moderate ($p = 0.61$) or severe ($p = 0.84$) subsets. After Bonferroni correction for multiple comparisons, none of the results remained significant.

Trans-ethnic meta-analysis. We performed a trans-ethnic meta-analysis on 5268 subjects (three European ($n = 4710$) cohorts combined with one African ($n = 245$) and one Admixed American ($n = 313$) cohort). We considered only variants present in all five cohorts, analysing 6318669 imputed autosomal variants. Study-level Manhattan plots are given in the Supplementary Fig. 2. The results were combined with meta-regression (genomic inflation factor, $\lambda = 0.939$). We found no genome-wide significant association with TBI outcome (Supplementary Fig. 5), but 40 variants attained sub-genome-wide significance ($p < 10^{-5}$) in eight loci (Table 3). As the sample consists of $\sim 90\%$ of Europeans, the results are mainly driven by individuals of European ancestry (Supplementary Table 6). However, of these, four loci were not present in the results of the European-specific meta-analysis.

Transcriptome-wide association study

The 4710 individuals of European ancestry were included in the TWAS and GREx could be imputed using PrediXcan for a total of 15104 unique genes across 15 GTEx tissue types (Supplementary Table 2). No

single gene reached significance at a transcriptome-wide level of $p < 0.05$ after Bonferroni adjustment for the number of comparisons within each tissue. TWAS Manhattan plots by tissue are given in Figure 2. A list of the top three associations per tissue are given in the Supplementary Table 7. Of these, *LINC00957* is an intergenic variant of unclear biological function. Of the remaining potential hits, *AKR1E2* polymorphisms have been indirectly linked to sleep duration (*rs7896356*)²⁹ and mathematical ability (*rs12773994*);³⁰ *TBX6* has been indirectly linked to multiple sclerosis (*rs3809627*);³¹ *DOC2A*, which is mainly expressed in the brain, and is involved in calcium-dependent neurotransmitter release, has been indirectly associated with autism and schizophrenia (*rs11646127*, *rs12691307*, *rs3814881*).^{32–34}

A focused analysis of 28 candidate genes previously associated with TBI did not link any of the candidates to the sliding dichotomy outcome via gene expression (Supplementary Table 8).

Discussion

We report the first GWAS and TWAS on TBI outcome. The largest genetic study of TBI to date, it leverages methodological approaches that enhanced power, including harmonized outcomes, inclusion of important covariates to mitigate variations in injury severity, and use of a sliding dichotomy to enable inclusion of all TBI severities. Finally, we comprehensively assessed

Locus	Lead variant	Chr	Position (Hg38)	Alleles (EA/OA)	Fixed-effects			
					EAF	log OR	SE	
COL24A1	rs2390015	1	86101570	C/A	0.485	0.218	0.045	1.34×10^{-6}
TBC1D8	rs6543009	2	101047475	C/T	0.797	-0.269	0.056	1.72×10^{-6}
ACT26121.3	rs9841867	3	59422182	C/T	0.091	0.367	0.080	4.23×10^{-6}
LINC01206	rs13072112	3	181984927	G/T	0.054	-0.522	0.112	3.18×10^{-6}
RIOK2	rs12521707	5	97461553	A/G	0.471	0.211	0.045	2.53×10^{-6}
FTMT	rs4562069	5	121641383	A/G	0.724	0.228	0.051	8.73×10^{-6}
ACO08695.1, RAIPGEF6	rs147117425	5	131554665	A/G	0.018	-0.810	0.180	6.36×10^{-6}
BLOC155-TXNDCS	rs2748364	6	7972244	C/G	0.490	-0.202	0.045	8.18×10^{-6}
HLA-DQB1	rs9274194	6	32662912	C/T	0.240	-0.280	0.061	4.86×10^{-6}
AL357507.1	rs12196383	6	74097727	C/A	0.037	0.668	0.141	2.23×10^{-6}
SLC29A4	rs12216559	7	5259270	T/C	0.425	0.221	0.046	1.46×10^{-6}
PSD3	rs57435548	8	18484796	T/C	0.615	-0.215	0.046	2.51×10^{-6}
MYO1D	rs1047208	17	32493413	T/A	0.394	-0.219	0.047	3.73×10^{-6}

Table 2: Loci attaining sub-genome-wide significance ($p < 10^{-5}$) in association with TBI outcome in European ancestry meta-analysis ($n = 4710$).

Chr: chromosome; EA: effect allele; OA: other allele; EAF: effect allele frequency; log OR: logarithm of odds ratio; SE: standard error of log OR.

prior published candidate gene associations in order to produce the most definitive assessment to date of the role of genetic variation in TBI outcome.

Our genome-wide estimate of heritability is consistent with a contribution of common genetic variation to inter-individual variability in TBI outcome. The estimated effect size ($h^2_{SNP} = 0.26$) is comparable with the heritability of risk of neurological and neuropsychiatric traits and diseases,^{35–39} many of which share phenotypic characteristics and, perhaps, common biology with TBI. While the precise measure of heritability is likely to change with larger sample sizes and evaluation of more precise phenotypic measures such as imaging variables, our result provides strong motivation for a continued search for genetic variants that alter individual responses to TBI.

While we identified some variants and genes that met sub-genome-wide significance thresholds for an association with TBI outcome, none achieved thresholds for genome-wide significance using either GWAS or TWAS. Despite inclusion of 5268 TBI patients, our cohort was likely insufficiently powered to detect variants associated with outcome variability. This analysis and the overall simple and straightforward strategy used, from the definition of the trait to the analysis settings, lays the groundwork for inclusion of additional cohorts, providing the power needed to understand the impact of genetics in such a heterogeneous disease. Indeed, substantially larger GWAS analyses promoted by international consortia have been required for identification of variants associated with complex disorders such as stroke,⁴⁰ psychiatric disorders,⁴¹ epilepsy,³⁸ and Alzheimer's disease.⁴²

We did not replicate prior reported associations with TBI outcome for most variants previously studied in smaller candidate gene studies. Of note, we found a weak association between carriage of an *APOE* ϵ_4 allele and outcome in a secondary analysis. We identified a single variant in *MBL2* (rs1800450; C>T), which reached statistical significance after correction for multiple testing in the current analysis, and was associated with better outcomes. The association of this mutation is biologically plausible, since the variant is associated with lower levels of mannose-binding lectin⁴³ (MBL) and susceptibility to autoimmune disease and infections, and has been associated with improved outcome in ischaemic stroke.⁴⁴ The remaining three variants that reached nominally significant associations with outcome (uncorrected $p < 0.05$) were also in inflammatory host response genes: rs361525 in the *TNF* gene (associated with higher levels of *TNF* α);⁴⁵ rs5030737 and rs7096206 in the *MBL* gene (associated with low MBL levels).^{46,47} The presence of neuroinflammation in response to TBI has already been extensively described and characterized using different *in vivo* and *in vitro* models.^{48–50} Additional analyses focusing on pathways involved in neuronal immune response, as

Locus	Lead variant	Chr	Position (Hg38)	Alleles (EA/OA)	MR-MEGA	
					EAF	p-value
<i>COL24A1</i>	rs2390015	1	86101570	C/A	0.467	1.09×10^{-6}
<i>BHLHE40*</i>	rs2163909	3	5044114	A/G	0.441	1.51×10^{-6}
<i>FHIT</i>	rs113548485	3	59415748	T/C	0.081	2.47×10^{-6}
<i>EIF4G1*</i>	rs56148883	3	184333858	A/G	0.082	9.56×10^{-6}
<i>BLOC155-TXNDC5</i>	rs1150893	6	7967713	T/C	0.531	7.69×10^{-6}
<i>PSD3</i>	rs57832455	8	18484815	G/C	0.585	2.71×10^{-6}
<i>MBL2*</i>	rs11003134	10	52778399	A/C	0.138	4.14×10^{-6}
<i>PIGH*</i>	rs2319844	14	67598317	T/C	0.809	1.42×10^{-6}

Table 3: Loci attaining sub-genome-wide significance ($p < 10^{-5}$) in association with TBI outcome in trans-ethnic meta-regression (n = 5268).

Chr: chromosome; EA: effect allele; OA: other allele; EAF: effect allele frequency.

* New loci compared to European ancestry meta-analysis (Table 2).

well as on other mechanisms involved in TBI pathophysiology,⁵¹ will be required to further investigate the genetic and biological background underlying TBI outcome, potentially unraveling new drug targets for TBI treatment. The lack of validation of most candidate variants is consistent with the well-established limitations and interpretation of candidate gene studies.⁵² However, these results do not exclude a role for candidate gene studies where strong biological evidence exists. Due to technical limitations, such as its absence from the genotyping array used, limited imputation accuracy, or MAF <1%, we were not able to address the impact of rs333, the 32bp deletion on *CCR5* gene,⁵³ or variants in *TRPM4*, which have been associated with TBI outcome and lesion progression.

A transcriptome-based analysis yielded no statistically reliable new associations with TBI outcome. Our negative result is most likely due to inadequate power, but could alternatively indicate that environmental factors, rather than genetic predisposition, dominate the regulation of the relevant expression levels.

We did not undertake HLA-specific genotype imputation,⁵⁴ also expression imputation was limited in this region. This limits any conclusions we can draw about outcome associations of the HLA region, and thus candidate interleukin genes.

We acknowledge that using GCS for defining inclusion and substratification is not perfect. However, it does provide direct translatability to disease constructs understood by clinicians, and allowed us to map onto past studies of genetic associations of TBI outcome. We considered using CT classifications as covariates of interest, but chose not to do so, since many CT features (including the extent of intracranial haemorrhage and severity of oedema, and hence the presence of mass effect) are plausibly on the mediation path of genetic drivers of outcome. The exploration of differential effects on different TBI subtypes would have been interesting, but were constrained by sample size and consequent statistical power.

While GOSE provides an excellent summary measure of overall functional outcome, the level of recovery achieved by any individual is dependent on a complex mixture of physical disability, mental health sequelae, and cognitive deficits, and is modulated by variable access to rehabilitation. The use of endpoints such as depression, anxiety, and post-traumatic stress disorder provide opportunities for more refined analyses that focus on individual facets of outcome. When data are available, subsequent analyses will involve measurable intermediate phenotypes such as lesion progression on quantitative CT imaging, admission levels and dynamic patterns of protein biomarkers in blood, or changes in brain volume and structure. These intermediate phenotypes may be more strongly related to quantitative trait loci, and provide a more tractable initial approach to understanding the impact of host biology on disease progression and outcome in TBI. We elected to assess GOSE at 6 months, as this is the conventional time point for outcome assessment in TBI, and the primary endpoint in our contributing studies. However, we recognise longer term outcomes, as well as the trajectory of recovery beyond six months would have been interesting to study.

The use of a multi-state model for imputation allowed us to expand sample size and increase study power, but we need to recognise that, even using this approach, we had to exclude patients in whom no follow up was possible. These patients were not missing completely at random, and their exclusion needs to be taken into account when considering the generalisability of our results.

We chose to implement a logistic regression for genome-wide single-marker analysis. Although multi-level regression models might provide an unbiased estimate of standard errors, given the nested data structure, we elected not to use this approach. The simpler logistic regression model was less complex to implement, more easily scalable for further studies, and expected to provide unbiased point estimates of effects despite some

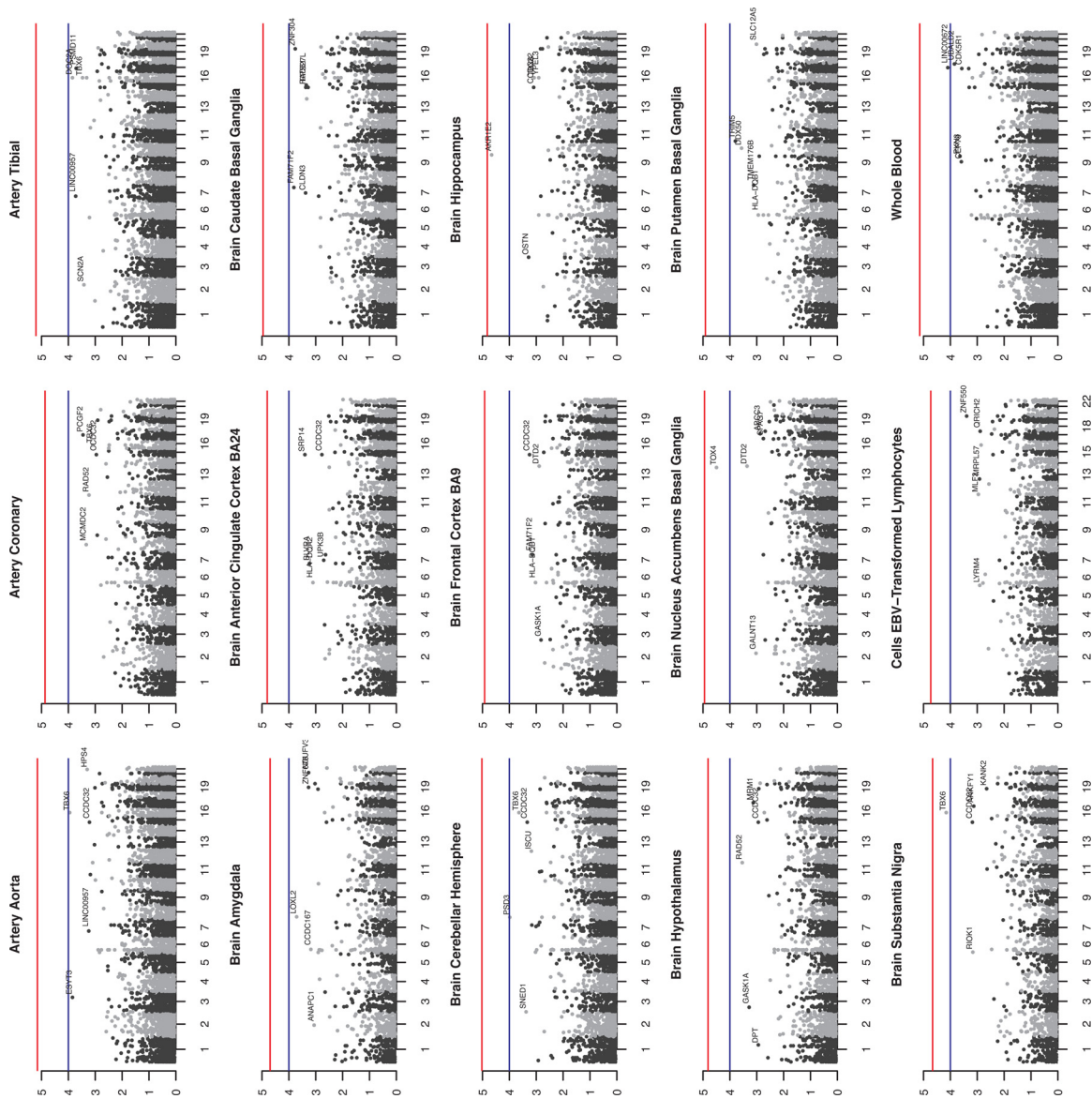


Figure 2. Manhattan plots of European ancestry TWAS by tissue for TBI outcome ($n = 4710$). Red lines correspond to tissue-wide significance under Bonferroni correction, blue line is fixed at a p-value of 10^{-4} .

underestimation of standard errors for the variance component.

Despite the limitations of this analysis, the GAIN consortium has provided the foundation for a meticulous and complex process of phenotype harmonization across the different study sites, as well as a synchronized and collaborative analysis of the genetic data. Building on this pivotal effort, we were able to demonstrate a substantial genetic component of TBI through our heritability analysis and to show that the detailed mapping of the complex genetics underlying TBI are yet beyond the reach of even this collaboration. The

generation of this large TBI international patient cohort, the standardized collection and curation of traits of interest, as well as the setting up of a standardized analytical and meta-analytical pipeline are therefore only a first crucial step that lays the basis for the inclusion of additional TBI cohorts in future TBI GWAS studies. We would argue that all substantive ongoing TBI studies, both observational and interventional, which collect key injury covariates and good quality outcome data, should be encouraged to use harmonizable common data elements, bank DNA, and obtain consent for subsequent collaborative analyses using the approaches we have

described. The incremental costs of banking DNA are minimal, and those of genotyping rapidly falling. Establishing a sample size of tens of thousands of TBI patients with fully harmonized (or harmonizable) data and outcomes would provide a strong basis for leveraging funding for genotyping and analysis.

In conclusion, the international collaborative effort promoted through the GAIN initiative has allowed us to perform the first GWAS and TWAS analyses of TBI outcome, providing the foundation for future analyses. The inclusion of additional cohorts, as well as the analysis of additional traits, will furthermore clarify the role of genetic factors underlying the observed phenotypic variability, potentially identifying still unraveled therapeutic targets.

Contributors

The full details of author contributions are listed in the Supplementary file Contributors.

The following authors have verified the underlying data:

Genotype data: Kals M, Kunzmann K, Parodi L, Radmanesh F, Rosand J, and Ripatti S

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All authors read and approved the final manuscript.

Declaration of interests

K.K. is now an employee of Boehringer Ingelheim. S.I. declares royalties or licenses with McGraw Hill education. L.W. reports receiving consultancy fees from Vasopharm and Novartis outside the submitted work. C.D.A. declares sponsored research support from Bayer AG and consulting fees from ApoPharma. R.D.A. declares consultation for MesoScale Discoveries and Ischemix, Inc.; stocks/stock options in BrainBox Solutions, Inc. and NovaSignal, Inc.; and has received equipment, materials, drugs, medical writing, gifts or other services from MesoScale Discoveries. M.B.S. declares advisory work and stock options with Oxeia Biopharmaceuticals. A.I.R.M. serves as an advisory board member for PressuraNeuro. D.K.M. declares the following conflicts of interest outside the scope of the submitted work: collaborative grant funding, consultancy fees, or educational grants from Lantmannen AB, GlaxoSmithKline Ltd., PressuraNeuro Ltd., Calico LLC, NeuroTrauma Sciences LLC, and Integra Neurosciences. He acts as a Trustee for Queens' College (Cambridge, UK), the Intensive Care National Audit and Research Centre (London, UK), and is Chair and Trustee of the European Brain Injury Consortium. J.R. declares participation on advisory boards for Takeda Pharmaceuticals, Pfizer and Boehringer Ingelheim, outside the scope of the submitted work. The remaining authors declare no competing interests.

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Data sharing statements

GWAS summary statistics from the European ancestry and the trans-ancestry meta-analysis and TWAS summary statistics are made available through zenodo.org (DOI: 10.5281/zenodo.5826420).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ebiom.2022.103933](https://doi.org/10.1016/j.ebiom.2022.103933).

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