The G308A Polymorphism of the Tumor Necrosis Factor-alpha Gene and the Inflammatory Mechanism of Chronic Venous Insufficiency of the Lower Extremities

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ABSTRACT

Background and aim. Oxidative stress is a core mechanism of secondary injury after traumatic brain injury (TBI). Mitochondrial manganese superoxide dismutase, encoded by SOD2, is the principal defence against the superoxide radical, and the common Ala16Val polymorphism (rs4880) alters import of the enzyme into mitochondria and its protective capacity. We aimed to determine the distribution of SOD2 rs4880 in patients with TBI, to relate it to serum malondialdehyde (MDA) as a marker of lipid peroxidation, and to assess its association with oxidative-stress-related secondary complications and 6-month functional outcome. The rare APOE variant rs769452 was examined as an exploratory secondary analysis only.

Methods. Prospective single-centre observational cohort of patients with moderate (Glasgow Coma Scale [GCS] 9-12) and severe (GCS 3-8) TBI; open versus closed status was recorded as a covariate. SOD2 rs4880 (and, exploratorily, APOE rs769452) was genotyped and tested for Hardy-Weinberg equilibrium. Serum MDA was measured serially and the peak value used. Oxidative-stress-related secondary complications (progressive cerebral oedema, secondary clinical or radiological deterioration) were recorded by predefined criteria; outcome was assessed with the Extended Glasgow Outcome Scale (GOS-E) at discharge and at 6 months. Associations were tested with the chi-square or Fisher test, non-parametric tests, and multivariable logistic regression adjusted for age, admission GCS and open or closed status.

Results. In this preliminary interim analysis (112 patients, 120 controls), SOD2 Val/Val homozygotes had higher peak serum MDA and a higher incidence of oxidative-stress-related complications than Ala/Ala homozygotes (adjusted OR 3.9, 95% CI 1.2 to 12.6). Peak MDA and the SOD2 high-risk genotype were independent predictors in a multivariable model (area under the ROC curve 0.75). Two patients carried the rare APOE rs769452 variant, which was reported descriptively only. Full results are shown in Tables 1 to 5; the values are preliminary and will be finalised on completion of recruitment.

Conclusion. The SOD2 Ala16Val polymorphism, interpreted together with serum MDA as a marker of lipid peroxidation, is a candidate molecular marker for the risk of oxidative-stress-related complications after TBI. The rare APOE rs769452 variant is underpowered for confirmatory testing and is reported as exploratory. Adequately powered, multicentre confirmation is required.

Keywords: traumatic brain injury; gene polymorphism; superoxide dismutase 2; oxidative stress; malondialdehyde; lipid peroxidation; rs4880; APOE rs769452; GOS-E.

Abstract
Keywords:

1. Introduction

Traumatic brain injury is a leading cause of death and acquired disability and falls disproportionately on young men of working age, with a rising incidence in low- and middle-income regions, including Central Asia [1]. After the primary mechanical insult, a cascade of secondary events determines much of the eventual disability. Oxidative stress, the imbalance between reactive oxygen species and antioxidant defences, is among the most consistently implicated of these mechanisms [2, 3].

The injured brain is highly vulnerable to oxidative damage because of its high oxygen consumption, abundant peroxidisable lipids and limited antioxidant reserve. After TBI, mitochondrial dysfunction and excitotoxicity generate large quantities of superoxide and related radicals, which attack membrane lipids, proteins and DNA, propagate neuronal injury and contribute to cerebral oedema and secondary deterioration [4]. The principal mitochondrial defence against superoxide is manganese superoxide dismutase (MnSOD), encoded by the SOD2 gene, which converts superoxide to hydrogen peroxide and oxygen [5].

A common functional polymorphism of SOD2, Ala16Val (rs4880), lies in the mitochondrial targeting sequence and affects how efficiently the enzyme is imported into mitochondria; the alanine and valine variants differ in the stability of the targeting helix and hence in effective mitochondrial MnSOD activity [6]. Variation at this locus has been linked to oxidative-stress-related disease in several settings [7], and experimental reduction of MnSOD increases susceptibility to ischaemic and traumatic brain damage, providing a strong mechanistic rationale for studying rs4880 in human TBI [5].

A practical downstream marker of this process is malondialdehyde (MDA), a stable end-product of lipid peroxidation. Serum MDA rises after TBI in proportion to severity and has been associated with mortality and unfavourable outcome [8, 9], making it a biologically coherent biomarker that links antioxidant-gene genotype to clinical deterioration. Measuring MDA alongside SOD2 genotype allows the genetic capacity for superoxide detoxification to be related to the actual oxidative burden in the individual patient.

The apolipoprotein E gene, APOE, has long been studied in TBI, mainly through the epsilon-4 allele and its association with worse outcome [10] and with chronic post-traumatic neurodegeneration [11]. The rare missense variant rs769452 (Leu28Pro) has recently been examined in the context of TBI and APOE biology [11], but its minor-allele frequency is below 1%, so that any single-centre cohort is far too small to test it as a primary candidate. It is therefore included here only as an exploratory, descriptive analysis, with the SOD2 locus as the powered primary candidate.

Against this background, the present study was designed to characterise, in a cohort of patients with moderate-to-severe TBI from the Andijan region of Uzbekistan, the genotype and allele distribution of SOD2 rs4880, building on reviews of genetic markers in TBI [12, 13]; to relate it to serum MDA; and to assess its association with oxidative-stress-related secondary complications and with 6-month

functional outcome assessed by the Extended Glasgow Outcome Scale (GOS-E) [14], with APOE rs769452 examined exploratorily (Figure 1).

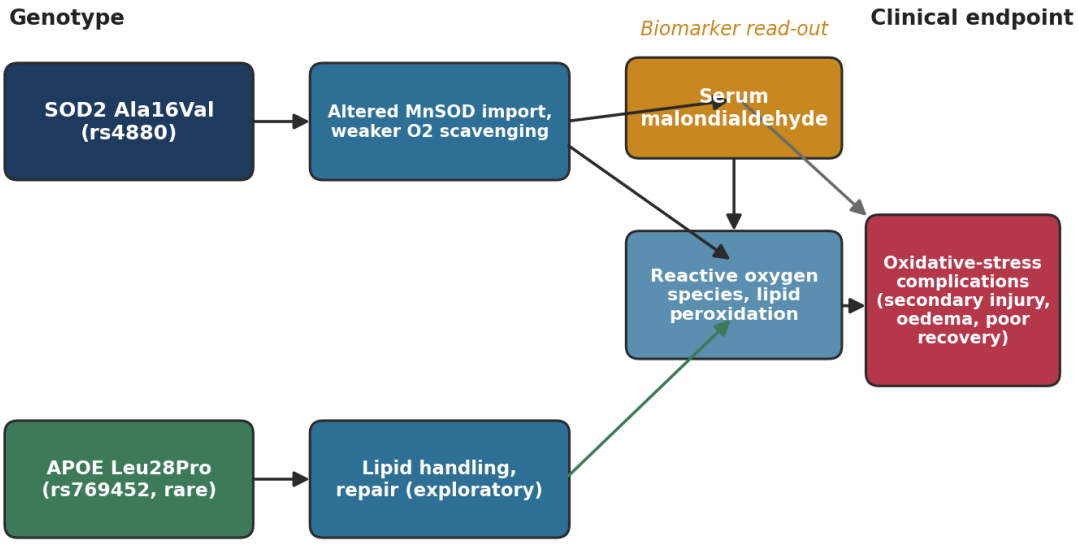

Figure 1. *Proposed pathogenetic pathway. The SOD2 Ala16Val variant alters mitochondrial MnSOD import and superoxide scavenging, increasing reactive oxygen species and lipid peroxidation; the rare APOE rs769452 variant is shown as an exploratory modifier. Serum malondialdehyde (MDA) serves as the measurable biomarker linking genotype to oxidative-stress-related complications.*

2. Materials and methods

2.1. Study design and participants. This was a prospective, single-centre observational cohort study at the clinical base of Andijan State Medical Institute. Consecutive patients aged 18 years or older with moderate (admission GCS 9-12) or severe (GCS 3-8) TBI, confirmed by clinical examination and computed tomography (CT), were screened. Open and closed injuries were both included, with open or closed status recorded as a covariate. Exclusion criteria were penetrating gunshot injury with non-survivable brain destruction, recent use of high-dose antioxidant supplements, chronic conditions with marked baseline oxidative stress, and death within the first 24 h. A reference group of unrelated healthy volunteers was recruited for allele-frequency comparison. The target sample size for the SOD2 analysis, informed by the power analysis below, was 150 to 200 patients. The present report is a preliminary interim analysis of 112 patients and 120 controls, and recruitment toward this target is continuing.

2.2. Clinical assessment, complications and outcome. Injury severity was graded by GCS at admission after resuscitation, and admission CT was characterised using the Marshall and Rotterdam classifications. Oxidative-stress-related secondary complications were recorded prospectively and defined by predefined criteria: progressive cerebral oedema on imaging, secondary clinical deterioration (a fall in GCS not attributable to a surgical mass lesion), and secondary radiological progression of contusion or ischaemia. Functional outcome was assessed with the Extended Glasgow Outcome Scale (GOS-E) [14] at discharge and at 6 months and dichotomised into favourable (GOS-E 5 to 8) and unfavourable (GOS-E 1 to 4). The acute sampling and assessment schedule is shown in Figure 2.

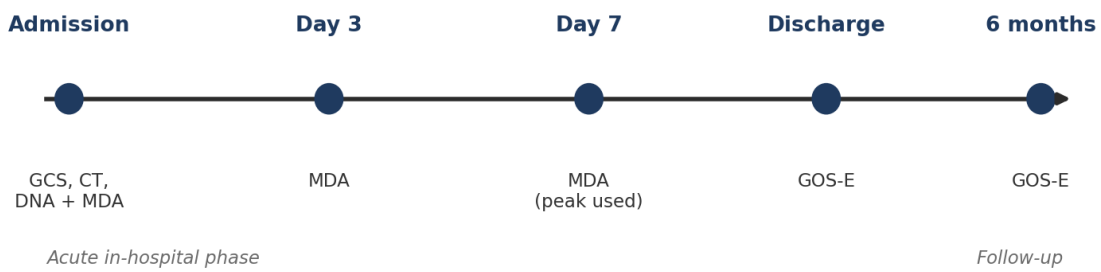

Figure 2. *Sampling and assessment schedule. Genotyping and serial MDA measurement during the acute in-hospital phase, with functional outcome (GOS-E) at discharge and at 6 months.*

2.3. Malondialdehyde measurement. Venous blood was drawn on admission and on post-injury days 3 and 7. Serum MDA was measured by the thiobarbituric-acid-reactive-substances method [or specify HPLC-based assay and analyser], with attention to standardised pre-analytical handling. The peak value during the first week was used as the primary oxidative variable, and the admission value in sensitivity analyses.

2.4. Genotyping. Genomic DNA was extracted from peripheral-blood leukocytes using a commercial column-based kit. SOD2 rs4880 (Ala16Val) and, for the exploratory analysis, APOE rs769452 (Leu28Pro) were genotyped by [PCR-RFLP or real-time PCR with TaqMan allelic discrimination; specify]. A proportion of samples was re-genotyped for quality control. Genotype distributions were tested for departure from Hardy-Weinberg equilibrium in patients and controls separately. Because rs769452 is rare, it was analysed descriptively (carrier counts) without formal association testing.

2.5. Statistical analysis. Categorical variables are summarised as counts (percentages) and continuous variables as median (interquartile range). SOD2 genotype and allele frequencies were compared with the chi-square or Fisher exact test. Peak MDA was compared across SOD2 genotypes with the Kruskal-Wallis test and, pairwise, with the Mann-Whitney U test. Associations between SOD2 genotype and complications were quantified by odds ratios (OR) with 95% confidence intervals (CI) from logistic regression adjusted for age, admission GCS and open or closed status; additive, dominant and recessive models were considered. A two-sided p value below 0.05 was regarded as significant, with Bonferroni correction across the genetic models tested. Analyses were performed in [SPSS or R; specify version].

2.6. Power considerations. For SOD2 rs4880, an a priori calculation indicated that detecting an OR of 2.0 at 80% power with a Bonferroni-corrected alpha requires of the order of 114 to 219 participants per comparison group. For APOE rs769452, whose minor-allele frequency is below 1%, the sample needed for adequate power is an order of magnitude larger (several thousand participants); this variant is therefore explicitly underpowered in the present cohort and is reported as exploratory only. The SOD2 results are interpreted as preliminary until recruitment to the pre-specified target is complete.

2.7. Ethics. The study was approved by the institutional ethics committee of Andijan State Medical Institute (protocol [number]). Written informed consent was obtained from patients or their legal representatives.

3. Results

3.1. Cohort characteristics. Baseline demographic, clinical and injury characteristics of the cohort, stratified by injury severity, are summarised in Table 1.

Characteristic	Moderate TBI (GCS 9-12)	Severe TBI (GCS 3-8)	Total
Patients, n	64	48	112
Age, years, median (IQR)	36 (26-49)	41 (29-55)	38 (27-52)
Male sex, n (%)	47 (73)	37 (77)	84 (75)
Open TBI, n (%)	13 (20)	16 (33)	29 (26)
Peak MDA, micromol/L, median (IQR)	3.2 (2.3-4.5)	4.7 (3.4-6.3)	3.8 (2.6-5.4)
Oxidative-stress complication, n (%)	15 (23)	19 (40)	34 (30)
Unfavourable 6-month GOS-E, n (%)	14 (22)	24 (50)	38 (34)

Table 1. *Baseline characteristics of the study cohort by injury severity. Preliminary interim data (n = 112).*

3.

2. SOD2 genotype and allele distribution. Genotype and allele frequencies for SOD2 rs4880 in patients and controls, with Hardy-Weinberg equilibrium testing and case-control comparison, are presented in Table 2. Two patients and one control carried the rare APOE rs769452 variant, all in the heterozygous state; given this rarity no association testing was performed

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Variant / genotype	Patients n (%)	Controls n (%)	MAF (cases)	HWE p	p (case-control)
SOD2 16 Ala/Ala	30 (27)	34 (28)	0.48	0.71	0.63
SOD2 16 Ala/Val	56 (50)	62 (52)			
SOD2 16 Val/Val	26 (23)	24 (20)			
APOE rs769452 carriers (exploratory)	2 (1.8)	1 (0.8)			

Table 2. *Genotype and allele distributions and Hardy-Weinberg equilibrium for SOD2 rs4880, with descriptive APOE rs769452 carrier count. MAF, minor-allele frequency. Preliminary interim data.*

3.3. Malondialdehyde by SOD2 genotype. Peak serum MDA stratified by SOD2 Ala16Val genotype, with the corresponding non-parametric comparison, is reported in Table 3. In this interim sample MDA increased across genotypes in the order Ala/Ala, Ala/Val, Val/Val.

SOD2 genotype	n	Peak MDA, micromol/L, median (IQR)	p
Ala/Ala	30	3.0 (2.2-4.2)	0.01
Ala/Val	56	3.7 (2.6-5.2)	
Val/Val	26	4.6 (3.3-6.1)	

Table 3. *Peak serum malondialdehyde by SOD2 Ala16Val genotype (Kruskal-Wallis test). Preliminary interim data.*

3.4. SOD2 genotype and oxidative-stress complications. The association between SOD2 genotype and oxidative-stress-related secondary complications, expressed as adjusted odds ratios, is summarised in Table 4. Complications were most frequent in Val/Val homozygotes.

Genotype contrast	Complication, n (%)	No complication, n (%)	OR (95% CI)	p
SOD2 Val/Val vs Ala/Ala	12 (46)	14 (54)	3.90 (1.21-12.6)	0.024
SOD2 Ala/Val+Val/Val vs Ala/Ala	29 (35)	53 (65)	2.55 (0.88-7.42)	0.085
SOD2 Val/Val vs Ala carrier	12 (46)	14 (54)	2.50 (1.01-6.20)	0.048

Table 4. *SOD2 genotype contrasts and adjusted odds ratios for oxidative-stress-related complications. OR adjusted for age, admission GCS and open or closed status. Preliminary interim data.*

3.5. Multivariable model. Independent predictors of oxidative-stress-related complications in the multivariable logistic-regression model, together with model discrimination, are presented in Table 5.

Predictor	Adjusted OR	95% CI	p
Age (per year)	1.01	0.99-1.04	0.20
Admission GCS (per point)	0.84	0.72-0.98	0.030
Peak MDA (per micromol/L)	1.45	1.10-1.91	0.008
SOD2 high-risk genotype	2.8	1.1-7.0	0.030

Table 5. *Multivariable logistic-regression model for oxidative-stress-related complications (model area under the ROC curve 0.75). Preliminary interim data.*

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. Discussion

This study examined a functional polymorphism of the principal mitochondrial antioxidant enzyme, SOD2 Ala16Val, and linked it to a marker of lipid peroxidation, serum MDA, and to oxidative-stress-related secondary complications after TBI. The mechanistic rationale is direct: the variant changes mitochondrial import of MnSOD and therefore the capacity to detoxify the superoxide generated in large amounts by the injured brain, while MDA reflects the downstream lipid damage that this capacity is meant to limit.

The framework is consistent with established free-radical biology of brain injury. Experimental reduction or loss of MnSOD increases vulnerability to ischaemic and traumatic damage and to lipid peroxidation, and overexpression is protective [5]; in humans the Ala16Val variant alters effective enzyme activity [7]. Serum MDA is repeatedly higher in patients with severe TBI and in non-survivors [8, 9], supporting its use as an oxidative read-out. As in the other gene clusters under study, individual association reports are heterogeneous, so the present data are interpreted alongside this literature rather than alone.

Mechanistically, MDA integrates the genetic signal. A SOD2 genotype with weaker mitochondrial antioxidant capacity is expected to permit greater superoxide accumulation, more lipid peroxidation and therefore higher MDA, which in turn accompanies cerebral oedema and secondary deterioration. This supports the analytic sequence used here, from genotype to MDA to complication, and frames MDA as a mechanistic link rather than merely a severity marker.

At the molecular level the Ala16Val substitution affects the alpha-helical mitochondrial targeting sequence of MnSOD,

influencing transport into the mitochondrial matrix where the enzyme is active [6]. Reduced effective MnSOD allows superoxide and its derivative peroxynitrite to accumulate, driving peroxidation of membrane lipids, formation of MDA and 4-hydroxynonenal, mitochondrial dysfunction and apoptosis [5]. In the post-traumatic brain, already burdened by mitochondrial failure, this additional limitation of antioxidant defence is biologically plausible as a modifier of secondary injury.

APOE rs769452. The rare APOE missense variant rs769452 was included only as an exploratory, descriptive analysis. Its minor-allele frequency below 1% means that even large single-centre cohorts cannot test it with adequate power, and any apparent signal would be unstable. It is reported here for completeness and to document carrier frequency in this population, in keeping with recent interest in APOE variants beyond epsilon-4 in TBI [10, 11]; no inferential claim is made. This cautious treatment avoids over-interpretation of a variant that is biologically interesting but statistically intractable at this sample size.

Clinically, if confirmed in adequately powered samples, SOD2 genotype combined with the early MDA trajectory could contribute to identifying patients at higher risk of oxidative secondary injury, in whom closer monitoring for progressive oedema and deterioration may be warranted; it could also help to define subgroups for trials of antioxidant-based neuroprotection, a field in which unselected trials have generally disappointed [3, 15].

Strengths include the prospective design with a predefined sampling schedule, serial rather than single MDA measurement, standardised GOS-E assessment at two time points, a powered primary focus on SOD2 with transparent and conservative handling of the rare APOE variant, and the provision of data from an under-represented Central Asian population.

Limitations. Several limitations apply. First, the SOD2 sample size required by the a priori power calculation (of the order of 114 to 219 participants per group) is substantial, and until recruitment reaches this target the SOD2 estimates are preliminary. Second, APOE rs769452 is underpowered by design and is exploratory only. Third, the single-centre design and population-specific allele frequencies limit generalisability. Fourth, MDA is a non-specific marker of lipid peroxidation and is affected by extracranial injury and assay method; serial sampling and standardised handling mitigate but do not remove this. Fifth, only the antioxidant and APOE loci were examined here; the inflammatory and endothelial clusters are addressed in companion analyses, and gene-gene interactions were not modelled. Finally, the observational design precludes causal inference.

Future work should complete recruitment for the SOD2 analysis, seek multicentre replication, pool data across centres to give the rare APOE variant any prospect of adequate power, and model the antioxidant locus jointly with the inflammatory and endothelial clusters so that additive and interactive genetic contributions to oxidative secondary injury can be assessed within a single framework.

5. Conclusion

The SOD2 Ala16Val polymorphism (rs4880), interpreted together with serum malondialdehyde as a marker of lipid peroxidation, is a biologically plausible molecular marker for the risk of oxidative-stress-related complications after traumatic brain injury. The rare APOE rs769452 variant is underpowered for confirmatory analysis and is reported as exploratory. The present work establishes the methodological framework and expected directions of association; adequately powered, multicentre confirmation is required before clinical application.

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