# Association Between Single Nucleotide Polymorphisms in *DNM1L* and Their Effect on Global and Functional Outcomes in Severe Traumatic Brain Injury

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Sustaining Brain Health Readiness against Emerging Threats in the Operational Environment ● MHSRS-26-5628

## Abstract

**Background:** Prognostication after severe traumatic brain injury (sTBI) remains challenging. Current multivariable models explain only a modest proportion of outcome variance, suggesting that additional biological determinants, including genetic variability, may contribute to recovery heterogeneity. *DNM1L* encodes dynamin-related protein 1 (Drp1), a key regulator of mitochondrial fission that links upstream calcium overload to downstream cytochrome c release and apoptotic commitment. Common *DNM1L* polymorphisms may therefore modulate the post-injury fission response, but their influence on recovery trajectories in adults with sTBI has not been characterized.

**Purpose:** The purpose of this exploratory study was to examine associations among *DNM1L* single nucleotide polymorphisms (SNPs), cerebrospinal fluid (CSF) biomarker indices of mitochondrial fission dynamics, and variability in global and functional outcomes at 3 and 6 months following sTBI.

**Methods:** The sample included 125 adults with sTBI (Glasgow Coma Scale [GCS] score 3–8) enrolled at a single academic Level I trauma center. Participants were predominantly male (78.4%) with a median age of 30 years, most (72.8%) carried the wild-type *DNM1L* genotype, and 76.0% experienced cerebral hypoxia (brain tissue oxygen tension < 20 mmHg). *DNM1L* genotype was determined using high-density array genotyping, with rs2272238 as the tagging SNP. CSF cytochrome c and calcium were quantified at 24- and 72- hours post-injury as mechanistically informed indices of the Drp1-mediated fission–apoptosis cascade. Bivariate analyses and multivariate models adjusted for age, sex, and GCS were used to evaluate associations between *DNM1L* genotype, hypoxic exposure, biomarker indices, and 3- and 6-month Glasgow Outcome Scale (GOS) and Disability Rating Scale (DRS) scores.

**Results:** *DNM1L* genotype was not associated with dichotomized global outcome (GOS). Linear mixed-effects modeling demonstrated a significant Genotype × Time interaction for DRS (p = .020), with SNP carriers exhibiting greater functional improvement between 3- and 6- months, which remained robust in a survivor bias sensitivity analysis (p < .001). Neither *DNM1L* genotype nor hypoxic exposure predicted CSF calcium or cytochrome c concentrations. However, a larger cytochrome c differential between 24- and 72- hours independently predicted early mortality (odds ratio = 4.05, 95% confidence interval [1.29, 12.73], p = .017).

**Conclusion:** These preliminary findings suggest that common *DNM1L* variation may contribute to heterogeneity in functional recovery after sTBI and that early cytochrome c variability is an independent predictor of mortality. Integration of mitochondrial fission genetics and dynamic biomarker indices into precision prognostic models warrants evaluation in larger, multicenter cohorts.

## Purpose, Specific Aims & Conceptual Model

**Purpose.** The purpose of this dissertation was to explore the association between single nucleotide polymorphisms (SNPs) in *DNM1L*, early CSF biomarkers of mitochondrial injury (cytochrome c and $Ca^{2+}$), and hypoxic burden on global and functional outcomes following severe traumatic brain injury (sTBI).

**Aim 1:** Compare 3- and 6-month global (GOS) and functional (DRS) outcomes between *DNM1L* genotype groups in adults following severe traumatic brain injury.

*Hypothesis: DNM1L SNPs may affect the function of the DRP1 protein promoting dysfunctional mitochondrial fission processes and increase the likelihood of poor outcome or death.*

**Aim 2:** Compare concentrations of cytochrome c and calcium ($Ca^{2+}$) from CSF between *DNM1L* genotypes with and without hypoxic burden.

*Hypothesis: Hypoxia triggers mitochondrial fission, potentially further exacerbating aberrant fission processes in DNM1L variant carriers. Variant carriers with greater hypoxic burden are therefore expected to exhibit more pronounced early CSF calcium depletion with altered recovery over 72 hours, accompanied by a larger rise in cytochrome c between 24 and 72 hours, reflecting amplified Drp1-mediated fission and apoptotic signaling.*

**Aim 3:** Explore the association of *DNM1L* genotype and hypoxic burden on global and functional outcomes at 3- and 6 months post-injury.

*Hypothesis: Carriers of the DNM1L SNP genotype, when coupled with elevated hypoxic burden, are expected to exhibit greater risk of poor global and functional outcomes as a result of impaired mitochondrial response to injury.*

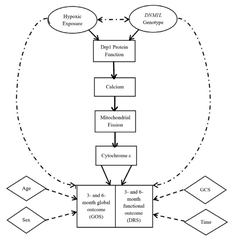



**Figure 1.** *A priori conceptual model. Solid arrows = fission cascade; dash-dot = hypothesized gene–environment interaction and direct genotype effect on recovery; dashed = precision covariates (age, sex, GCS, time). Early mortality examined post-hoc (not shown). Source: dissertation Figure 1.*

## Methods

**Design & sample.** 125 adults with severe TBI (GCS 3–8) with EVD and Licox monitoring at a single Level I academic trauma center.
**Genotype.** Four DNM1L SNPs, in near-complete linkage disequilibrium ($r^2$ = 0.98–1.00), formed a single haplotype block; genotype classified as wild-type vs. SNP carrier (tagging SNP rs2272238).
**Exposure & biomarkers.** Hypoxic Exposure ($PbtO_2$ < 20 mmHg, dichotomized as present/absent); CSF cytochrome c and calcium concentrations at 24- and 72-hours post-injury.
**Outcomes.** Global outcome — dichotomized Glasgow Outcome Scale and Functional outcome — continuous Disability Rating Scale (DRS), both at 3- and 6-months post-injury. Post hoc Early Mortality — dichotomized in-hospital mortality ≤ 22 days post-injury.
**Analysis.** Generalized estimating equations (GEE) modeled GOS and Early Mortality; linear mixed-effects models (LMM) modeled DRS and CSF concentrations of cytochrome c and calcium. Genotype, time, Hypoxic Exposure, and their interactions as predictors. Models were adjusted for age, sex, and GCS.

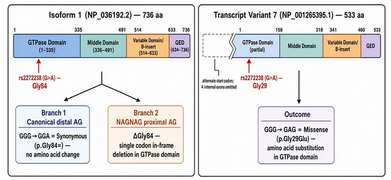
**Table 1. Sample characteristics and descriptive statistics (N = 125).**

| Characteristic | n (%) or M (SD) |
|---|---|
| Enrolled | 125 adults with severe TBI (GCS 3–8) |
| Age, years | M = 35.26 (SD = 16.51); Mdn 30, range 16–74 |
| Sex | Male 98 (78.4%) · Female 27 (21.6%) |
| Race | Caucasian 124 (99.2%) |
| GCS (enrolling) | Mdn 6 (IQR 5–7); GCS 6–8 81 (64.8%) · GCS 3–5 44 (35.2%) |
| **DNM1L genotype** | **Wild-type 91 (72.8%) · SNP carrier 34 (27.2%)** |
| **Hypoxic Exposure** | **Hypoxic 95 (76.0%) · Normoxic 30 (24.0%)** |
| Mortality | Early Mortality (≤ 22 d) 21 (16.8%) · Total through 6 mo 22 (17.6%) |
| Outcomes assessed | GOS 117 (93.6%) / 95 (76.0%) · DRS 114 (91.2%) / 95 (76.0%) at 3 / 6 mo |

*Notes: GCS = Glasgow Coma Scale; GOS = Glasgow Outcome Scale (dichotomized: poor 1–3, good 4–5); DRS = Disability Rating Scale (0–30, higher = greater disability); $PbtO_2$ = brain tissue oxygen tension. No baseline variable had missing data; missingness was confined to follow-up outcomes. Source: dissertation Table 1.*

## Genomic & Regulatory Context — *DNM1L* Haplotype

**Figure 2. Transcript-specific consequences of rs2272238 (G>A).**




**In brief.** One G>A change, three possible protein outcomes — silent, a one-glycine in-frame deletion (ΔGly84) in canonical isoform 1, or p.Gly29Glu in transcript variant 7: the consequence depends on which transcript is expressed.

**Detail.** Isoform 1 (NP_036192.2) — the canonical distal AG acceptor gives GGG→GGA (p.Gly84=), while the NAGNAG proximal AG gives a single-codon in-frame deletion (ΔGly84) in the GTPase domain. Transcript variant 7 (NP_001265395.1) begins at an alternate start codon (4 internal exons omitted), so the same base change is missense: GGG→GAG, p.Gly29Glu. Source: dissertation Figure 2.

**Table 2. *DNM1L* haplotype: functional annotation and brain sQTL effects (GTEx v10).**

| SNP | GRCh38 position | Functional annotation | Study MAF | Brain sQTL NES |
|---|---|---|---|---|
| rs7954206 | 12:32691772 | Intronic/regulatory variant | .150 | +0.36 to +0.53 |
| rs11052184 | 12:32695419 | Intronic/regulatory variant | .148 | +0.36 to +0.53 |
| **rs2272238** | **12:32707368** | **Transcript-dependent coding/splicing variant:** synonymous in canonical transcripts; NAGNAG-mediated ΔGly84; p.Gly29Glu in transcript variant 7 | **.149** | **+0.36 to +0.53** |
| rs10844314 | 12:32718547 | Intronic variant | .149 | +0.37 to +0.53 |

**Key finding.** The four SNPs travel as a single tightly linked haplotype block ($r^2$ = 0.98–1.00; D′ = 1.00), so they are statistically indistinguishable; rs2272238 is the functional candidate.

*Notes: MAF = minor allele frequency; NES = normalized effect size. Significant brain sQTL signals occur in nucleus accumbens, anterior cingulate cortex, cerebellum, and cerebellar hemisphere; positive NES indicates increased alternative splice-junction usage. Source: GTEx Portal v10 (2024).*

## Results — Global Outcome (GOS)

*DNM1L* genotype was **not significantly associated** with dichotomized global outcome (GOS) at either timepoint (p = .287); the Genotype × Hypoxic Exposure interaction was also non-significant (p = .294). Age and initial GCS remained the significant predictors of poor global outcome.

**Table 3. GEE results predicting poor global outcome (GOS 1–3), Aims 1 & 3.**

| Predictor | B | p | OR | 95% CI for OR |
|---|---|---|---|---|
| DNM1L genotype | 1.153 | .287 | 3.17 | [0.38, 26.43] |
| Time (6 vs. 3 months) | −0.363 | .114 | 0.70 | [0.44, 1.09] |
| Hypoxic Exposure | 0.606 | .256 | 1.83 | [0.64, 5.20] |
| **GCS 3–5 vs. 6–8** | **0.939** | **.027** | **2.56** | **[1.11, 5.88]** |
| **Age (per year)** | **0.041** | **.005** | **1.04** | **[1.01, 1.07]** |
| Genotype × Time | −0.751 | .122 | 0.47 | [0.18, 1.22] |
| Genotype × Hypoxic Exposure | −1.215 | .294 | 0.30 | [0.03, 2.86] |

Notes: GEE with binomial distribution, logit link, AR(1) working correlation; adjusted for age, sex, and GCS. Bold = p < .05. Reference categories: genotype = wild-type, sex = female, GCS = 6–8, time = 3 months, Hypoxic Exposure = normoxic. In the Aim 1 model without Hypoxic Exposure terms, the genotype main effect was likewise non-significant (p = .290; Genotype × Time p = .133). Source: dissertation Tables 5 & 11.

## Results — Functional Outcome (DRS)

A model incorporating Hypoxic Exposure revealed a significant Genotype × Time interaction predicting DRS scores: ***SNP carriers showed more favorable functional outcomes than wild-type*** between 3 and 6 months, contrary to the a priori hypothesis. The Genotype × Hypoxic Exposure interaction in the same model was non-significant (B = −3.31, p = .445), indicating hypoxic burden did not significantly moderate this genotype-associated recovery pattern. This effect persisted in sensitivity analyses excluding mortality cases.

Genotype × Time coefficient — whole-model estimate, applies across both oxygenation strata

| | | |
|---|---|---|
| **Aim 1 LMM** | genotype × time model (dissertation Table 6) | B = −2.20 · SE = 0.93 · p = **.020** |
| **Aim 3 LMM** | adds Hypoxic Exposure + Genotype × Hypoxic Exposure (dissertation Table 13) | B = −2.25 · SE = 0.53 · p < **.001** |
| **Sensitivity** | mortality cases excluded (n = 104; dissertation Table 17) | B = −2.27 · SE = 0.53 · p < **.001** |

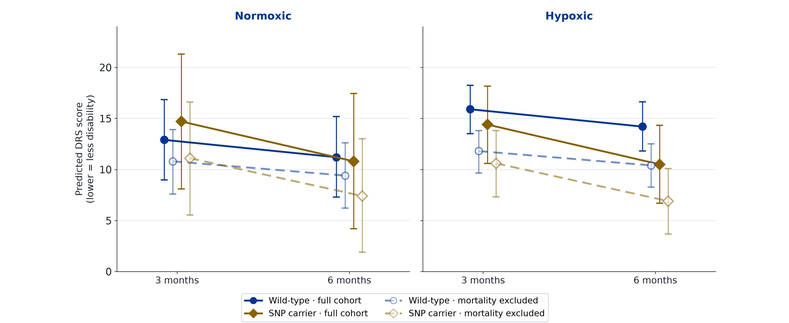



**Figure 3.** *Predicted DRS scores over time by DNM1L genotype and oxygenation group (full cohort vs. mortality excluded). Circles = wild-type; diamonds = SNP carrier. Solid = full-sample model; dashed = mortality-excluded model. Error bars = 95% CI. Lower DRS = less disability. Source: dissertation Table 18 / Figure 12.*

**Table 4. LMM results predicting DRS (Aim 3, fully adjusted).**

| Predictor | B | p |
|---|---|---|
| DNM1L genotype | 1.83 | .633 |
| **Time (3 → 6 months)** | **−1.48** | **< .001** |
| Hypoxic Exposure | 2.99 | .171 |
| **Genotype × Time** | **−2.25** | **< .001** |
| Genotype × Hypoxic Exposure | −3.31 | .445 |

**Interpretation.** Estimated marginal means indicate SNP carriers appeared ***buffered against Hypoxic Exposure***: wild-type participants under Hypoxic Exposure sat roughly 3 DRS points higher (greater disability) than normoxic wild-type participants at both timepoints (d = 1.36), whereas SNP-carrier DRS scores were nearly identical across oxygenation strata (ΔDRS < 0.3, d = 0.14). The interaction term itself did not reach significance, so this pattern is descriptive. Source: dissertation Tables 13 & 14.

## Results — CSF Biomarkers

Neither genotype nor Hypoxic Exposure significantly predicted CSF cytochrome c or calcium concentrations at 24 or 72 hours, and **none of the interaction terms reached significance**. Cytochrome c declined modestly over time in both genotype groups.

**Table 5. LMM results for CSF cytochrome c (representative biomarker model).**

| Predictor | B | p |
|---|---|---|
| DNM1L genotype | 0.29 | .490 |
| Hypoxic Exposure | 0.14 | .563 |
| **Time (24 → 72 h)** | **−0.36** | **.015** |
| Genotype × Hypoxic Exposure | −0.33 | .483 |
| Genotype × Time | 0.04 | .808 |
| Hypoxic Exposure × Time | 0.22 | .179 |

*Notes: LMM with per-participant random intercept, centered age as covariate. Calcium models were likewise null (genotype p = .871; Hypoxic Exposure p = .539; all interactions p ≥ .512). Source: dissertation Tables 7 & 9.*

## Post-Hoc Analysis — Early Mortality

In a post-hoc analysis prompted by a bimodal outcome distribution, greater variability in the absolute value of the change in concentration of cytochrome c between 24- and 72-hours post-injury independently predicted Early Mortality.

**|Δ Cytochrome c| per 1 ng/mL** — adj. OR = **4.05** 95% CI [1.29, 12.73] · p = **.017**

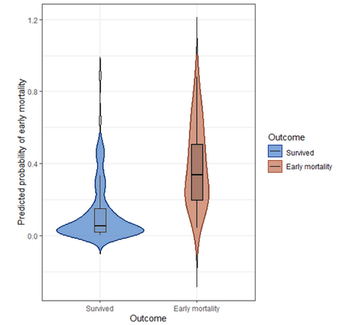



**Table 6. Post-hoc GEE model of early in-hospital mortality.**

| Predictor | OR | 95% CI |
|---|---|---|
| **\|Δ Cytochrome c\|** | **4.05** | **[1.29, 12.73]** |
| **GCS 3–5 vs. 6–8** | **6.15** | **[1.62, 23.28]** |
| **Age (per year)** | **1.08** | **[1.04, 1.12]** |

*Bold = 95% CI excludes 1. Predicted probability of early mortality: survivors (n = 104) M = 0.12 (SD 0.16), Mdn 0.05 [IQR 0.02–0.15]; early mortality (n = 21) M = 0.39 (SD 0.22), Mdn 0.34 [IQR 0.20–0.51]. Hypoxic Exposure × |ΔCytC| interaction non-significant (OR 0.52, p = .504). Source: dissertation Tables 15 & 16.*

**Figure 4.** *Distribution of model-predicted probabilities of early mortality by outcome group (survivors n = 104; early mortality n = 21), adjusted for Hypoxic Exposure, |ΔCytC|, DNM1L genotype, age, sex, and GCS. Distributions overlap in the lower range; wide intervals reflect limited power (21 deaths). Reproduced from dissertation Figure 11.*

## Discussion & Conclusions

### Interpretation & conclusions

- This exploratory study did not confirm the hypothesized deleterious effect of DNM1L variation on recovery. Instead, SNP carriers showed more favorable functional outcomes than wild-type on the DRS.
- A plausible mechanism is GTEx splicing quantitative trait locus (sQTL) evidence that this haplotype is associated with altered Drp1 isoform balance in brain tissue, shifting fission dynamics toward a more adaptive profile during subacute recovery.
- The absence of genotype or Hypoxic Exposure effects on CSF biomarkers may reflect limited statistical power from the pronounced hypoxic/normoxic imbalance (76% / 24%) rather than a true absence of biological interaction.
- That cytochrome c variability — rather than directionality or absolute concentration — predicted early mortality parallels established variability-based prognostic frameworks in cardiovascular and endocrine medicine.
- Genotype was not associated with dichotomized GOS or with absolute biomarker levels, supporting continuous outcome measures and serial biomarker sampling in future TBI prognostic models.
- These findings are hypothesis-generating and require replication in larger, ancestrally diverse cohorts before *DNM1L* genotype or biomarker instability can inform clinical prognostic models.

### Limitations

- Modest single-center sample (N = 125; 34 SNP carriers), 99.2% European ancestry and 78.4% male — limiting power for Genotype × Hypoxic Exposure and ancestry-stratified analyses.
- Hypoxic Exposure, and severity thereof, was structurally imbalanced (76% hypoxic / 24% normoxic); a binary $PbtO_2$ threshold cannot capture cumulative burden.
- Two CSF timepoints (24 and 72 h) and 6-month follow-up cannot capture full biomarker trajectories or longer-term recovery divergence.
- Functional annotation was in-silico (GTEx sQTL) without direct experimental validation.
- The mortality analysis was post-hoc and underpowered (21 deaths), with some wider confidence intervals; unmeasured pharmacologic and surgical exposures may contribute residual confounding.
- Findings derive from a civilian severe blunt-force TBI cohort; the mitochondrial secondary-injury framework is shared across brain-injury contexts but requires confirmation in military-relevant populations.

## Acknowledgments

With gratitude to my dissertation committee, the University of Pittsburgh School of Nursing, the University of Pittsburgh Department of Neurological Surgery, and the study participants and their families. Genotyping and CSF biomarker analyses were conducted within the parent TBI biobank cohort, University of Pittsburgh IRB STUDY19030228, PI: Ava M. Puccio.

**Key references:** Stromberg 2008 & Dijkland 2020 (IMPACT/CRASH prognostic models) · Fischer 2016 (Drp1 fission in TBI) · Sridharan 2024 (Fis1–Drp1 blockade) · Conley 2014 (mitochondrial polymorphisms and TBI outcomes) · Au 2012 & Wagner 2013 (CSF cytochrome c) · Okonkwo 2017 ($PbtO_2$ threshold) · Karczewski 2020 (gnomAD) · GTEx v10 (brain sQTL)