Genetic variants in the circadian rhythm pathway predict posttraumatic stress symptom severity following trauma

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INTRODUCTION

- Persistent posttraumatic stress symptoms (PTSS) are common after motor vehicle collision (MVC) and major thermal burn injury (MThBI)[1]
- Previous literature suggests that alterations in circadian rhythm (CR) signaling influence PTSS vulnerability. For example, blunted rhythmicity of sleep/wake cycle indicators[2] and core circadian rhythm genes/gene regulators[3,4,5] (e.g., *RORA*, *CLOCK*, and *TEF)* have been shown to predict PTSS severity.
- Previously literature also suggests that CR pathway influence depends on stress severity and/or vulnerability. [6] A well-studied genetic factor contributing to stress vulnerability is *FKBP5* rs3800373 allele.

HYPOTHESES

- Genetic variants in CR pathway genes predict PTSS severity following MVC and MThBI.
- The influences of CR genes on PTSS depends on level of individual peritraumatic distress and individual *FKBP5* rs3800373 allele.

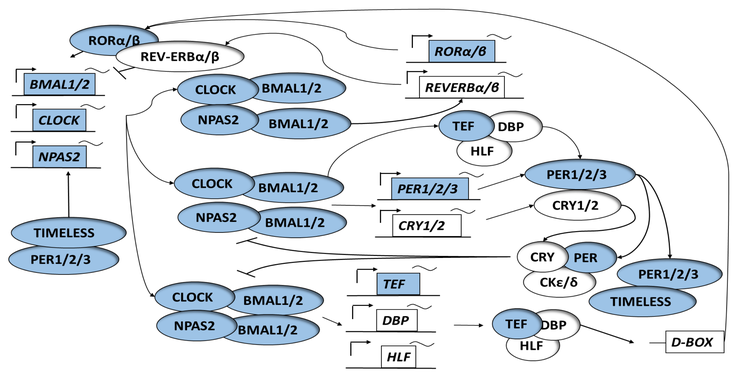
METHODS

African American men and women ≥18 and ≤ 65 years of age presenting to one of 13 different emergency departments (EDs) within 24 hours of MVC who did not have a serious fracture or other injury requiring hospital admission were enrolled (n = 907). This MVC cohort served as the discovery cohort. For the replication cohort, African American and European American men and women ≥18 and ≤ 61 years of age were enrolled after MThBI (n = 68). DNA samples were (PAXgene) collected in the immediate aftermath of trauma. PTSS severity was assessed using the Impact of Events Scale Revised (IES-R, MVC cohort) or the PTSD Symptom Scale Interview (PSS-I, MThBI cohort) 6 weeks, 6 months, and 1 year following trauma. Peritraumatic distress was assessed using the Peritraumatic Distress Inventory (PDI). Samples were genotyped using the MEGA platform (Illumina). Repeated measure mixed modeling adjusted for age, sex, study site, and time following trauma was used to evaluate associations between PTSS severity following MVC and 31 common genetic across 9 CR-pathway genes (*PER3, NPAS2, PER2, CLOCK, RORB, BMAL1, TIMELESS, RORA,* and *TEF*). Potential SNP*sex, SNP*distress, and SNP**FKBP5* SNP interactions were also evaluated. To account for multiple comparisons, we adjusted p values using the False Discovery Rate. RNA samples (RNA PAXgene tubes) were collected in the ED. To evaluate influence of *TEF* variant on gene expression, total RNA was library prepped using Ovation Human Blood RNA-Seq Library Systems kit (NuGen) and sequenced on a HiSeq 2500. Raw sequencing reads were aligned to hg19 using STAR, quantified using RSEM, and normalized to the overall upper quartile. RNA*SNP*PDI interactions were assessed using linear regression models adjusted for age, sex, and study site.

TABLE 1. Study participant characteristics.

Characteristic	MVC	MThBI
Participants, n	907	68
Females, n (%)	570 (62%)	18 (26%)
Age, years, mean (SD)	35.06 (12.62)	37.61 (12.15)
ED Stress Level, mean (SD)	22.26 (11.57)	13.06 (11.94)
BMI, mean (SD)	29.93 (7.62)	28.31 (6.99)
Education, n(%)		
High school or less	366 (40.22%)	32 (47.06%)
Post high school training other than college	41 (4.51%)	2 (2.94%)
Some college	333 (36.59%)	23 (33.82%)
College	134 (14.73%)	8 (11.76%)
Post-College	36 (3.96%)	3 (4.41%)

FIGURE 1. The core circadian rhythm pathway.



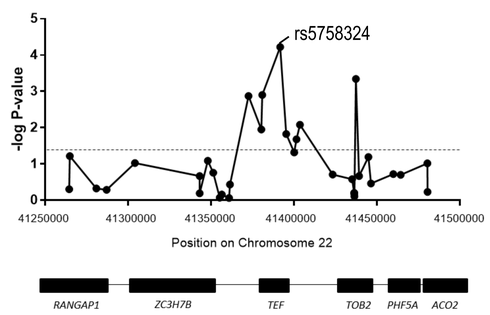
Genetic variants in genes highlighted in blue were the focus of this study.

TABLE 2. Five genetic variants from four circadian rhythm-associated genes predict posttraumatic stress symptom (PTSS) severity following motor vehicle collision.

Gene Name	SNP	Alleles	MAF	Interaction	p-value*	FDR corr. p-value
BMAL1	rs969485	A/G	0.480	-	$6.12*10^{-4}$	$2.016*10^{-3}$
RORA	rs4774388	T/C	0.222	sex	$4.79*10^{-4}$	$1.210*10^{-3}$
NPAS2	rs12622050	G/A	0.289	peritraumatic distress	$5.61*10^{-4}$	$1.613*10^{-3}$
TEF	rs5758324	T/G	0.295	peritraumatic distress	$6.47*10^{-5}$	$8.065*10^{-4}$
TEF	rs738499	T/G	0.150	peritraumatic distress	$1.38*10^{-3}$	$2.419*10^{-3}$
TEF	rs738499	T/G	0.150	*FKBP5*	$2.92*10^{-5}$	$4.032*10^{-4}$

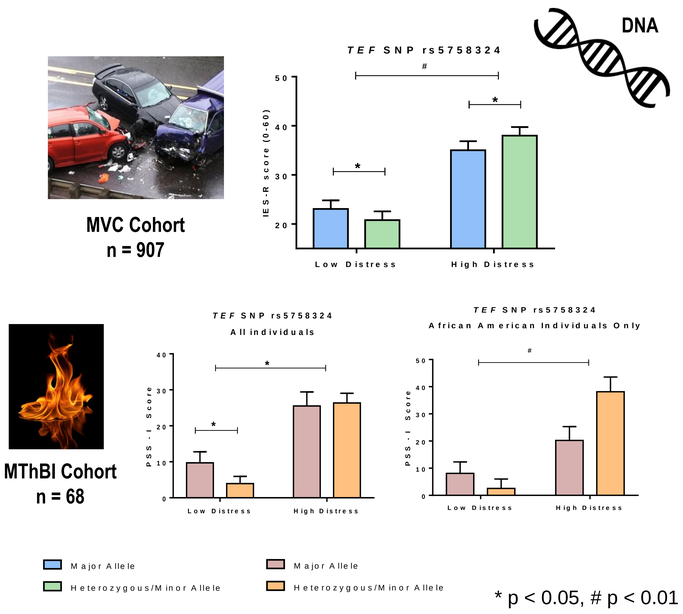
*p value generated via repeated measures mixed models (6WK, 6M, 1YR), adjusted for age, sex, study site, and time following MVC. The FDR corrected p value was calculated based on 124 association tests (31 SNPs each tested for association with PTSS as a main effect and interactions with sex, distress, and *FKBP5* SNP rs3800373).

FIGURE 3. Association between SNPs in the *TEF* genomic region and PTSS severity following MVC.



-log p-values represent SNP*stress interaction significance from repeated measures mixed models (6WK, 6M, 1YR) adjusted for age, sex, study site, and time following MVC.

FIGURE 2. *TEF* SNP rs5758324 interacts with stress to predict PTSS severity following MVC. This finding replicated in a second cohort of individuals experiencing major thermal burn injury (MThBI).



* p < 0.05, # p < 0.01

FIGURE 4. Among individuals with high distress in the early aftermath of MVC who develop substantial PTSS severity, *TEF* mRNA expression levels are lower in individuals with the rs5758324 major allele and higher in individuals with the rs5758324 minor allele.

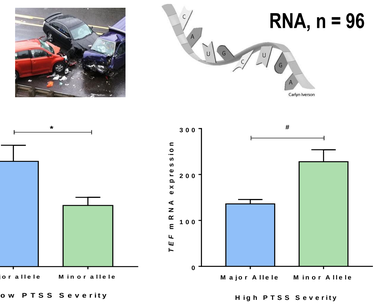
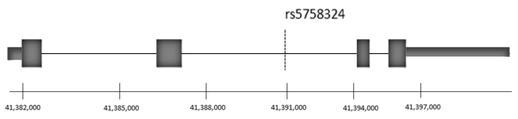


FIGURE 5. SNP rs5758324 is located in Intron 2 of the *TEF* gene



RESULTS

- Characteristics of study participants are provided in Table 1.
- 31 genetic variants from 9 CR genes were assessed (Figure 1).
- 5 SNPs from 4 CR-associated genes were associated with PTSS severity following MVC (False Discovery Rate < 5%): *BMAL1* (rs969485, $p=6.12x10^{-4}$); *RORA* (rs4775351*sex, $p=4.79x10^{-4}$); *NPAS2* (rs12622050*stress, $p=5.61x10^{-4}$); *TEF* (rs5758324*stress, $p=6.47*10^{-5}$; rs738499*stress, $p=1.38*10^{-3}$) (Table 2).
- Thyrotroph embryonic factor (*TEF)* polymorphism rs738499 also predicted PTSS severity in a *FKBP5* vulnerability allele-dependent manner (rs738499**FKBP5*rs3800373, $p=2.92x10^{-5}$) (Table 2).
- Stress-dependent associations for *TEF* alleles rs5758324 and rs738499 replicated in the MThBI cohort ($p=2.89x10^{-4}$, p=0.043, respectively) (Figure 2).
- Polymorphisms in and near the *TEF* gene exhibited the strongest stress-dependent association with PTSS severity following MVC, with rs5758324 the most significant (Figure 3).
- A significant interaction between *TEF* RNA expression levels, distress, and rs5758324 was identified (Figure 4).
- rs5758324 is located in Intron 2 of the *TEF* gene (Figure 5).

CONCLUSIONS

The results of our study suggest that genetic variants involved in the core circadian rhythm pathway predict PTSS severity after MVC and MThBI. In particular, *TEF* genetic variant rs5758324 predicted PTSS severity in a stress-dependent manner in both cohorts. The effect of this SNP on RNA expression levels suggest that it may be functional. Future studies investigating the role of *TEF* in PTSS pathogenesis are warranted.

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