Association between blood p11 and PTSD in Service Members

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Abstract

Stressful life events-induced over expression of the p11 (S100A10), a member of the S100 protein family that represents the largest group within the EF-hand super-family of Ca2+ binding proteins, has been observed in the brain of both rodent and humans. However, the association of blood p11 with post-traumatic stress disorder (PTSD), a traumatic event-related disorder has not been explored. PTSD is a debilitating mental disorder with a prevalence of more than 7% in the US population and 12% in the military. To begin intervention at the earliest possible time, priority must be given to identify a biomarker determining the presence of PTSD. Thus, translational research using cutting edge technology and skills to develop a blood biomarker to detect PTSD at its earliest and most treatable stage would benefit both providers and patients. Previously, we found that brain p11 mRNA was significantly over expressed by glucocorticoid in stressed rodent and post mortem cortex of patients with PTSD. Here we reported a study in which we hypothesize that there is association of PTSD with blood p11 and measured p11 plasma level in 67 subjects with PTSD and in 67 without in US service members deployed to Iraq and Afghanistan, a high-risk military population. We also examined the association between probable PTSD and three single-nucleotide polymorphisms (SNPs; rs2338019, rs3791153, and rs11204922) covering the p11 gene in the service members (n=3937). No association between these SNPs and PTSD was found. The linear regression analysis showed that blood p11 levels was positively correlated with of PCL (PTSD check list) score (r = 0.31, F=0.00027), and among the PTSD, the scores of PTSD symptom clusters re-experience was also positively associated with blood p11 (r =0.26, F=0.0023). Also, the blood p11 level was higher in PTSD than in controls (P=0.0002). Our data suggests that p11 protein may be involved in the pathophysiology of PTSD, serving as a potential biomarker for PTSD that merits investigation especially in military population.

Materials and Methods

Participants: Between November 2009 and July 2014, 3937 volunteers were enrolled from active duty US Army Special Operations Command and National Guard units who had been deployed in Iraq and Afghanistan. The average age of the subjects with or without PTSD were 36.5 and 36.2 years, respectively. Current PTSD symptoms were assessed using the PTSD Checklist (PCL), a 17-item Diagnostic and Statistical Manual Fourth Edition (DSM-IV) based self-report measure with well-established validity and reliability. The total PCL score determined PTSD symptom severity. Probable PTSD was determined based on endorsement of DSM-IV criteria and PCL score ≥ 44. Lifetime traumatic events or trauma exposure were assessed using the Life Events Checklist (LEC), a commonly used self-report measure assessing experiences that meet the DSM-IV PTSD definition of a traumatic stressor. Based on the PCL score, 67 participants were identified as possible PTSD from 889 Fort Bragg participants who donated blood samples. Therefore, 67 gender- and education-matched participants were selected as non-PTSD controls for blood p11 measurement.

Saliva sample collection, DNA extraction and genotyping

Saliva samples were collected using Oragene™ DNA Self-Collection Kits according to the manufacturer's instructions (DNA Genotek). Saliva DNA was extracted using the manufacturer's protocol. All genotypes were discriminated with 7900HT Fast Real-Time PCR system using the TaqMan 5'-exonuclease assay. The primers of p11. The total 5-µl of PCR mixture contained 5-ng DNA, 120-nM ADP 1× Master Mix (ABI), and 1× SNP assay. Amplification conditions were 2 min at 50° C, 10 min at 95° C, and then 40 cycles at 96° C for 15 s and at 62.5° C for 90s. Genotypes were generated using the ABI PRISM 7900 Sequence Detection system software. To evaluate genotyping accuracy, one-quarter of the samples, randomly selected, were genotyped in duplicate. The error rate was <0.005, and the completion rate was >0.95 (Applied Biosystems).

Plasma collection and plasma concentration of p11 assay

8.5 ml venous blood was collected with BD Vacutainer glass whole blood tubes (Product #: 364606. BD, New Jersey). The whole blood samples were centrifuged at 1300 x g for 10 minutes at 4 degrees. And then the plasma was transferred to a labeled fresh Eppendorf tube and stored at -80° C. Plasma levels of p11 were determined by highly sensitive immunoenzymatic assays (ELSIA), following the procedure suggested by the manufacturer (S100A10 elisa kit, Cat No. MBS591230. MYBioSource, San Diego, CA, USA). A monoclonal anti-human-p11 antibody was used. p11 concentration was determined from the regression line for the p11 standard curve (ranging from 7.8 to 500 ng/ul) conducted under similar conditions in each assay.

Statistics

All analyses were performed with SPSS version 18. Student t-tests were performed in order to examine the age difference between the PTSD and non-PTSD. Liner regression was conducted to examine the relationships between p11 and PTSD as well as severity of PTSD syptomes. The independent variable was considered as a statistically significant at a coefficient p-value less than 0.05.

Results

Table 1. Demographic information

		Controls	PTSD	P value
Age		29.3±8.4	30.1±8.3	0.09
Sex				
	Male	3525	350	0.81
	Female	504	52	
Ethnic				
	AAN	55	11	0.24
	API	473	53	
	Black	223	23	
	White	2808	291	

AAN: American Indian or Alaskan Native, API: Asian or Pacific Islander

Table 2. Demographic information for those with p11 measurement

		Controls	PTSD	P value
Age		27.1 ±6.0	26.5 ±6.2	0.29
Sex				
	Male	69	68	0.79
	Female	8	9	

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The genotype distribution of three p11 SNPs in the samples of NG , guam, and fort bragg

			Genotype			
Gene	SNPs	Phenotype	11	12	22	P value
p11	rs2338019	Controls	541	1913	1035	0.0904
		PTSD	59	189	79	
	rs3791153	Controls	258	1412	1921	0.624
		PTSD	25	145	176	
	rs11204922	Controls	1031	1570	649	0.094
		PTSD	106	150	45	

Figure 1.

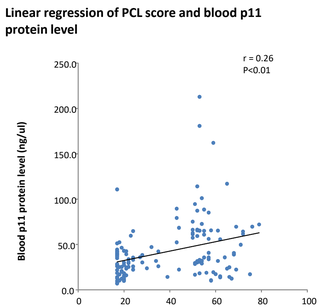
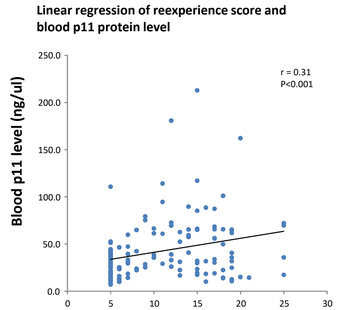
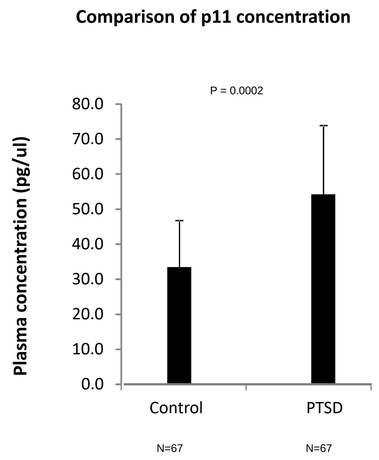


Fig 2





Conclusion

Our data suggests that p11 protein may be involved in the pathophysiology of PTSD, serving as a potential biomarker for PTSD that merits investigation especially in military population.