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GENES ENCODING THE COMPLEMENT ALTERNATIVE PATHWAY FACTORS IN POSTTRAUMATIC STRESS DISORDER

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Introduction

Posttraumatic stress disorder (PTSD) is a complex psychiatric disorder (DSM-IV-TR code: 309.81 [1]; ICD-10 codes: F43.1, F62.0 [2]). PTSD is an anxiety disorder developed in a person experiencing, witnessing, or learning about an extreme physically or psychologically distressing event [1, 2]. Results of epidemiologic, clinical and experimental studies suggested implication of both environmental and genetic factors in pathogenesis of PTSD [3-5]. Whereas the environmental factors triggering PTSD are well defined, less is known about PTSD-associated unfavorable genetic conditions.

Promising studies suggest the involvement of alterations in the immune status, particularly development of low-grade inflammatory reactions, in the pathogenesis of PTSD [6-9]. Our own studies have revealed PTSD-associated changes in the major mediator of the inflammatory-immune responses, the complement system. These changes were, in particular, detected at the level of the activity of the complement alternative pathway [10-12]. To understand whether the detected alterations are genetically determined or conditioned by other external/internal environmental factors, in the present study we have evaluated functional single nucleotide polymorphisms (SNPs) of genes encoding factors B, H and I, in patients with PTSD and healthy subjects (controls). Factor B is an essential component of the complement alternative pathway, and factors H and I are negative regulators of this pathway [13-15].

Materials and Methods

In the present study 150 PTSD affected subjects (males 129, females 21; mean age±SD: 42±11.3), Karabakh combat veterans within 16 years from traumatic event were examined. All the affected subjects were hospitalized at the Stress Center. Diagnosis of PTSD was determined by the Structured clinical interview for diagnostic and statistical manual of mental disorders (patient version) [16] and the clinician administered PTSD scale (CAPS) [17]. Age- and sex matched healthy controls (n=226; males 190, females 36; mean age±SD: 39±9.1) were volunteers without any history of physical or sexual abuse or other major trauma, defined as being free of current or past psychiatric disorders as determined by structured clinical interview (non-patient version) [18].The exclusion criteria were participant reports on any serious disease with genetic pre-

Table 2
Selected SNPs and relevant primers

Gene	SNP ID	Type and nucleotide sequence (5′→3′) of primer
CFB	rs12614	for standard allele: ACTCCATGGTCTTTGGCCC for mutant allele: ACTCCATGGTCTTTGGCCT constant: TGAGTCTTCAGGGTGCTCC
	rs1048709	for standard allele: GGGTACTGCTCCAACCCG for mutant allele: GGGTACTGCTCCAACCCA constant: GGACGATCTTCCGCTTCTGT
CFH	rs424535	for standard allele: GAGAACAGCAGCAGAGGAAA for mutant allele: GAGAACAGCAGCAGA GGAAT constant: GCCTGGTAAACAATGCCTCT
	rs800292	for standard allele: CCTTCCTGCATACAATTATTA for mutant allele: CCTTCCTGCATACAATTATTAT constant: GACCTGTGACTGTCTAGGC
	rs1061170	for standard allele: CCCTGTACAAACTTTCTTCCATA for mutant allele: CCCTGTACAAACTTTCTTCCATG constant: CTTAGTAACTTTAGTTCGTCTTCAG
CFI	rs10033900	for standard allele: CTATGTGACAGAGACCAGGA for mutant allele: CTATGTGACAGAGACCAGGG constant: ACCTTCAGGCGAGCTCACA
	rs1000954	for standard allele: AATAGTAGGCATCAGAGAAGTC for mutant allele: AATAGTAGGCATCAGAGAAGT T constant: CTAGGC TACTTGAAGTTGCC
	rs4469075	for standard allele: CCAGGGTGTGTGATG GGTC for mutant allele: CCAGGGTGTGTGATG GGTG constant: GAGGAGAGGTAC TGCCAG C

disposition. All subjects were Armenians born and living in Armenia. Both, the informed consents from all study subjects and the approval of the Ethics Committee of the Institute of Molecular Biology (IRB #00004079) were received for these studies.

About 5 ml of peripheral venous blood was collected from each study subject and transferred to EDTA-containing tubes. Genomic DNA samples were isolated from fresh blood according to previously described method [19] and stored at -30°C until use. Using polymerase-chain reaction with sequence-specific primers (PCR-SSP) DNA samples were genotyped for *CFB* rs12614 (C/T), rs1048709 (G/A); *CFH* rs424535 (T/A), rs800292 (C/T), rs1061170 (C/T); *CFI* rs10033900 (T/C), rs1000954 (G/A), rs4469075 (C/G) functional SNPs as described earlier [20]. Brief characteristics of studied genes are given in Table 1. Selected SNPs and relevant primers are listed in Table 2.

Table 1
Brief characteristics of the studied genes

Gene		
name	**ID**	**localisation on chromosome**
CFB	629	6p21.3
CFH	3075	1q32
CFI	3426	4q25

SNPs were selected based on either their functionality according to the National Center of Biotechnology Information (NCBI) databases (http://www.ncbi.nlm.nih.gov/) or tagging results obtained using the International HapMap Project database (http://hapmap.ncbi.nlm.nih.gov). All primers for PCR-SSP were designed using the genomic sequences in the GenBank nucleotide sequence database (https://www.ncbi.nlm.nih.gov/genbank/). The presence/absence of allele-specific amplicons in the PCR products was visualized in 2% agarose gel stained with ethidium bromide fluorescent dye using DNA molecular weight markers as a reference. To check the reproducibility of results, randomly selected DNA samples (10% of total) were genotyped twice.

Distributions of genotypes of the *CPLX2* rs3892909 and rs1366116 SNPs were checked for correspondence to Hardy-Weinberg equilibrium. To evaluate a potential link between these tagged SNPs and SCZ, their genotype, allele (gene), and phenotype frequencies (carriage rates) in SCZ-affected and control subjects were compared. To determine the significance of differences in genotype, allele, and phenotype frequencies between the study groups, the odds ratio (OR), 95% confidence interval (CI), and p-value were calculating using Pearson's chi-square test. The Bonferroni correction was used to correct P-values in multiple comparisons [21]. Statistical power was estimated according to the earlier described protocol [22]. Corrected P-values < 0.05 were accepted statistically significant. The data was analyzed using GraphPad Prism 6.0 application package (GraphPad Software Inc., USA).

Results and Discussion

Frequency distributions of genotypes for the selected SNPs of the studied genes in both PTSD-affected and control groups were consistent with the Hardy-Weinberg equilibrium (p>0.05). In Table 3-5 the allele and phenotype frequencies and mutant allele carriage rates of the selected SNPs in PTSD-affected subjects and controls are indicated. According to obtained results significant difference in the above mentioned parameters was detected only in case of CFI rs1000954 SNP (p<0.05). Here, a negative assotiation

Table 3
Genotype and allele frequencies of CFB SNPs in PTSD patients and controls

GENE SNP ID	Genotype (%)		Allele (%)		Mutant allele carriers (%)	
	PTSD	Controls	PTSD	Controls	PTSD	Controls
CFB rs12614	CC 58.4 CT 39.6 TT 2.0	CC 55.3 CT 39.6 TT 5.3	C 78.2 T 21.8	C 74.9 T 25.1	41.6	44.7
p =			0.32		0.56	
OR [95% CI]			0.84 [0.59-1.19]		1.13 [0.75-1.72]	
CFB rs1048709	GG 91.8 GA 7.5 AA 0.7	GG 92.3 GA 7.7 AA 0.0	G 95.5 A 4.5	G 96.0 A 4.0	8.0	7.7
p =			0.7		0.94	
OR [95% CI]			1.16 [0.54-2.50]		0.94 [0.42-2.09]	

Table 4
Genotype and allele frequencies of CFH SNPs in PTSD patients and controls

GENE SNP ID	Genotype (%)		Allele (%)		Minor allele carriers (%)	
	PTSD	Controls	PTSD	Controls	PTSD	Controls
CFH rs800292	GG 80.0 GA 17.0 AA 3.0	GG 74.0 GA 24.0 AA 2.0	G 89.0 A 11.0	G 86.0 A 14.0	20.0	26.0
p =			0.28		0.16	
OR [95% CI]			0.78 [0.50-1.23]		1.43 [0.87-2.37]	
CFH rs1061170	TT 21.0 TC 36.0 CC 43.0	TT 10.7 TC 46.2 CC 43.1	T 39.0 C 61.0	T 33.8 C 66.2	47.0	56.9
p =			0.17		1.0	
OR [95% CI]			0.78 [0.60-1.10]		1.92 [1.05-3.50]	
CFH rs424535	AA 38.0 AT 32.0 TT 30.0	AA 34.4 AT 50.2 TT 15.4	A 54.0 T 46.0	A 59.5 T 40.5	62.0	14.0
p =			0.145		0.47	
OR [95% CI]			1.25 [0.93-1.69]		1.17 [0.76-1.80]	

Table 5
Genotype and allele frequencies of *CFI* SNPs in PTSD patients and controls

GENE SNP ID	Genotype (%)		Allele (%)		Minor allele carriers (%)	
	PTSD	Controls	PTSD	Controls	PTSD	Controls
CFI rs10033900	TT 25.5 TC 41.6 CC 32.9	TT 31.0 TC 44.0 CC 25.0	T 46.3 C 53.0	T 53.7 C 47	74.5	69.3
p =			0.089		0.279	
OR [95% CI]			1.29 [0.962-1.73]		0.77 [0.486-1.23]	
CFI rs1000954	CC 66.0 CT 27.0 TT 7.0	CC 48.8 CT 43.6 TT 7.6	T 80.0 C 20.0	T 70.6 C 29.4	34.0	50.6
p =			0.02		0.006	
OR [95% CI]			0.61 [0.42-0.88]		2.03 [1.29-3.20]	
CFI rs4469075	CC 13.0 GG 42.0 GG 45.0	CC 10.4 GG 46.3 GG 43.3	C 34.0 G 66.0	C 33.5 G 66.5	55.0	56.7
p =			1.0		0.7	
OR [95% CI]			0.98 [0.7o-1.37]		1.32 [0.64-2.70]	

between the mutant allele of this SNP and PTSD was observed. Statistical power of this study reached 84%. In case of other selected SNPs of the studied genes, no significant association with PTSD was detected (p>0.05).

The complement system is a network of 35-40 soluble and membrane-bound enzymes, receptors, and regulators, a major effector on the innate and adaptive immunity, mediator of a variety of cellular and humoral interactions in the immune-inflammatory responses. Complement has an essential role in the immune recognition, defense against invading pathogens, transformed and damaged host cells, in maintaining immunologic memory, cell proliferation and differentiation, tissue regeneration, and aging. Mostly all tissues and organs, including brain, are able to produce the components [23-25]. Complement activation is known to occur through three pathways, classical, alternative, and lectin, differed in components and initiation mechanisms. Inappropriate complement activation contributes to pathophysiology of many diseases [26-27].

The complement factor I is a serine protease, an 88kDa heterodimeric glycoprotein composed of 2 polypeptide chains linked by disulfide bonds [28, 29]. Being negative regulator of the complement system it prevents uncontrolled deleterious activation of the complement through alternative pathway [13-15]. The gene encoding the complement factor I protein comprises 13 exons localized on chromosome 4q25 [30] and its deficiency is associated with a number of diseased conditions including recurrent severe infections, glomerulonephritis or autoimmune diseases, atypical haemolytic uremic syndrome, a severe disease characterized by thrombocytopenia, microangiopathic haemolytic anaemia and acute renal failure [31]. The rs1000954 SNP is localised in the intronic region of the *CFI* gene (http://diseasome.kobic.re.kr), and this is the first study evaluating the rs1000954 SNP in a diseased condition. Other SNPs of the *CFI* gene evaluated in this study, rs10033900 and rs4469075, have been shown to associate with choroidal neovascularization with high myopia in Europeans [32] and age-related mascular degeneration in Chinese, respectively [33].

Conclusions

In summary, we concluded that *CFI* represent a candidate gene for PTSD and that mutant allele of the *CFI* rs1000954 SNP may be considered a protective factor for this disorder.

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ԱՄՓՈՓՈՒՄ

Կոմպլեմենտի այլընտրանքային ուղու գործոնները կոդավորող գեները հետտրավմատիկ սթրեսային խանգարման ժամանակ

Ավետյան Դ.[1], Պետրոսյան Մ.[2], Հարությունյան Կ.[2], Մուսաելյան Ռ.[2], Հովհաննիսյան Լ.[1], Խանոյան Դ.[1], Մկրտչյան Գ.[1], Այվազյան Վ.[1], Բոյաջյան Ա.[1]

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Կոմպլեմենտի համակարգը հանդիսանում է բորբոքային իմունային պատասխանի կարևորագույն միջնորդներից մեկը: Մեր վերջին հետազոտությունները վկայում են այն մասին, որ հետտրավմատիկ սթրեսային խանգարման (ՀՏՍԽՄ) ժամանակ դիտվող կոմպլեմենտի այլընտրանքային ուղու ակտիվության փոփոխությունները ներգարավված են թույլ արտահայտված համակարգային բորբոքային գործընթացներում: Համաձայն ժամանակակից տեսության ՀՏՍԽՄ-ն հանդիսանում է պոլիգենային տիպի ժառանգական բազմագործոն բնույթի հիվանդություն: Այս հետազոտությունում մենք գնահատել ենք կոմպլեմենտի այլընտարանքային ուղու B, H և I գործոնների կոդավորող գեների ֆունկցիոնալ պոլիմորֆիզմների հնարավոր կապը ՀՏՍԽՄ-ի հետ:

РЕЗЮМЕ

Гены, кодирующие факторов альтернативного пути комплемента, при посттравматическом стрессовом расстройстве

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Система комплемента является одним из основных медиаторов воспалительного иммуного ответа. Результаты наших недавних исследований свидетельствуют о том, что нарушение альтернативного пути активации комплемента вовлечены в наблюдаемые при посттравматическом стрессовом расстройстве (ПТСР) вялотекущие системные воспалительные реакции. Согласно современным представлениям ПТСР относится к заболеваниям многофакторной природы с полигенным типом наследования. В настоящем исследовании мы оценили возможную связь функциональных полиморфизмов генов, кодирующие факторов альтернативного пути комплемента B, H и I, с ПТСР.