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Short communication

Genetic variants of the inflammatory C-reactive protein and schizophrenia in Armenian population: A pilot study

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Summary

C-reactive protein (CRP) is an inflammation marker implicated in the pathogenesis of schizophrenia. To investigate association of the *CRP* rs1417938, rs1800947, rs1205 variants with susceptibility to schizophrenia 208 unrelated Armenians (103 patients and 105 healthy controls) were genotyped. In this pilot study none of studied variants was associated with schizophrenia.

Introduction

Schizophrenia is a complex mental disease with genetic component characterized by a variety of psychotic symptoms, including delusions and hallucinations, altered emotional reactivity and disorganized behavior (Porteous *et al.*, 2008). Several possible mechanisms underlie this disease pathology such as altered DNA methylation (Feng & Fan, 2009), apoptotic engulfment pathway (Chen *et al.*, 2009), prefrontal-limbic and autoimmune dysregulation, abnormal glutamatergic transmission (Rădulescu, 2009), hyper- and hypofunction of dopaminergic system in brain different regions and microglial hypothesis (reviewed in Monji *et al.*, 2009). Importantly, many studies including findings from our group revealed immune system alterations in schizophrenia (Rădulescu, 2009; Mojni *et al.*, 2009; Boyajyan *et al.*, 2008; Bilbo & Schwarz, 2009).

C-reactive protein (CRP) produced by liver is an acute and chronic phase inflammation marker and plays an important role in the immune response. There is growing evidence on the implication of CRP in the pathogenesis of schizophrenia. Increased serum levels of high sensitivity C-reactive protein (hsCRP) in Arab schizophrenic patients were observed (Akanji *et al.*, 2009). Also, serum CRP levels were associated with the severity of cognitive impairment but not with psychiatric symptoms of schizophrenia (Dickerson *et al.*, 2009). C-reactive protein has also emerged as a target for cardiovascular (CV) risk outcomes in schizophrenia patients (Meyer *et al.*, 2009). Furthermore, CRP gene is located on the first chromosome, and a positive linkage of schizophrenia with chromosome 1q loci has been reported (Hennah *et al.*, 2006). During preparation of this article three-locus haplotypes [rs1417938 (A/T), rs1800947 (C/G) and rs1205 (C/T)] were generated by Halder et.al. and a positive association between Center for Epidemiological Studies-Depression (CESD) scores

and CRP levels among individuals with the A-G-T CRP haplotype has been found (Halder *et al.*, 2010). It has been shown recently that CRP polymorphisms are independently associated with increased (rs1205) and decreased (rs1800947) CRP level and that CRP genotypes/haplotypes interact with obesity to set CRP level (Teng *et al.*, 2009).

As inflammation is possible contributing factor in schizophrenia, the current study is aimed to investigate whether three selected genetic variants in the *CRP* gene are implicated in susceptibility to schizophrenia in Armenian population. The selection of investigated single nucleotide polymorphisms (SNPs) was based on their frequent occurrence in European population (www.pubmed.com) and their functionality. This is a pilot study concerning association of selected SNPs in the CRP gene with schizophrenia in Armenian population.

Materials and methods

Study population

A total of 208 unrelated Caucasian individuals of Armenian nationality were enrolled in this study. All 103 patients (mean age±SD: 46±9.88 years) were diagnosed as paranoid schizophrenics according to the International Classification of Disease (ICD-10) F20.0 criteria (http://www.who.int/classifications/icd/en/) by two independent experienced psychiatrists. The affected subjects were recruited from the Nubarashen Republic Psychiatric Hospital of the Ministry of Health of Armenia (MH RA). 105 ethnically matched healthy volunteers (37±11.32) without familial history of schizophrenia (control group) recruited from “Erebouni” Medical Center of MH RA were used as reference control population samples. Blood samples from patients and control subjects were collected between June and August 2009.

The study was approved by the Ethics Committee of Institute of Molecular Biology, National Academy of Sciences (NAS) of Armenia, Yerevan. All subjects signed informed consent about the usage of their blood samples for the research purposes of this study.

Methods

Genomic DNA extraction

Genomic DNA was isolated from fresh whole blood samples and was stored at -30°C until used for the genotyping. DNA extraction was performed according to the standard phenol-chloroform method (Sambrook & Russell, 2001).

Genotyping analysis

Three *CRP* SNPs, namely rs1417938, rs1800947, and rs1205, were genotyped using polymerase chain reaction with sequence-specific primers (PCR-SSP). The amplification reaction was carried out under the conditions described elsewhere (Bunce *et al.*, 1995). All primers were designed according to the *CRP* gene reference genomic sequences from the GenBank (http://www.ncbi.nlm.nih.gov, GeneID:1401). The primers' sequences were: 1) rs1417938: allele T, forward 5´CCC CCA TAC CTC AGA TCA AAT, allele A, forward 5´CCC CCA TAC CTC AGA TCA AAA, constant reverse 5´TCC AAA GGA GTG AAT TCA GGC; 2) rs1800947: allele C, forward 5´ GTG TTA ATC TCA TCT GGT GAC, allele G, forward 5´ GTG TTA ATC TCA TCT GGT GAG, constant reverse 5´AGT ACA CAT TTG TAC AAG CTG G; 3) rs1205: allele C, forward 5´AGT TTG GCT TCT GTC CTC AC, allele T, forward 5´ AGT TTG GCT TCT GTC CTC AT, constant reverse 5´GTG AAC CAC AGG GTG TCC. PCR products were visualized by electrophoresis on 2% agarose gel and ethidium bromide fluorescence in reference to a molecular weight marker. The genotyping was repeated for 10% of randomly selected samples (n=21) to check for confidence of the genotyping and in

each case concordant result was obtained.

Statistical analysis

The distributions of genotypes for all studied SNPs were checked for correspondence to the Hardy-Weinberg (H-W) equilibrium. Allelic (gene) and phenotype frequencies in the patients and control groups were compared. Allelic and phenotype frequencies were calculated according to the observed number of genotypes. Haplotype analyses were performed using SNP analyzer software (Yoo *et al.*, 2005). In order to reconstruct haplotypes in patients and controls groups Expectation-maximization (EM) algorithm was used (Excoffier & Slatkin, 1995). The extent of genetic association or linkage disequilibrium (LD) between different loci located in a specific chromosome was estimated by the same software. The odds ratio (OR), 95% confidence interval (CI) and Pearson's value (*p*-value) were calculated. The significance of differences between phenotype and allele frequencies in both groups was calculated using Pearson's Chi-square test (SPSS Inc, Chicago). Statistical power of the present study was calculated according to the protocol described elsewhere (Lalouel & Rohrwasser, 2002). *P* values less than 0.05 were considered as statistically significant.

Results and discussion

In order to reveal possible association between *CRP* gene variants and susceptibility to schizophrenia the groups of well characterized Armenian schizophrenics with ethnically matched controls were genotyped. The distribution of genotypes for all three investigated *CRP* SNPs corresponded to the Hardy-Weinberg equilibrium. Statistical power of the present study to detect the differences in the frequency of selected investigated allele (rs1417938*T) between the healthy controls and patients corresponding to the odds ratio 2 reached 96.2%.

The frequencies of all investigated *CRP* variants in patients with schizophrenia and control subjects are shown in Table 1. No significant differences were found when the proportions of *CRP* variants were compared between the patients and controls ($p>0.05$). Nevertheless, the rs1417938 AA homozygotes and rs1205*T carriers tended to be overrepresented in the patients by comparison with the control subjects ($p=0.1$ in both cases, Table 1).

There have been several levels of evidence that the expression and systemic levels of CRP may be under the genetic control of *CRP* gene polymorphism. Accordingly, all *CRP* gene variants investigated in the present study might be functional as suggested in the recent study by Halder *et al*. (2010). In their study, depressive symptomatology was measured using the Center for Epidemiological Studies-Depression (CESD) scale, and plasma CRP was assayed from whole blood. Three polymorphisms [rs1417938 (A/T), rs1800947 (C/G) and rs1205 (C/T)] were genotyped and three-locus haplotypes were generated. In regression models adjusting for age, gender, education, smoking status and statin use, one *CRP* haplotype (T-G-C) was associated with CRP level. Higher CESD scores were associated positively with CRP levels among individuals with the A-G-T haplotype ($p = 0.004$). It has been indicated that haplotypic variation of rs1417938, rs1800947 and rs1205 in the *CRP* gene moderated an association of depressive symptoms with circulating *CRP* (Halder *et al.*, 2010). Another study in Finnish population confirmed the association between prostate-specific antigen (PSA) and *CRP* rs1800947 variant in prostate cancer (PC). *CRP* alleles previously found to protect against increased CRP levels were suggested to be associated with metastatic PC, indicated by elevated PSA (Eklund *et al.*, 2009).

The observation of elevated CRP levels has been reported as evidence for an inflammatory component of schizophrenia and as a marker of more severe clinical symptoms and psychopathology in schizophrenics (Mazzarello *et al.*, 2004; Fan *et al.*, 2007). Furthermore, high sensitivity C-reactive protein *(hsCRP)* levels in serum of Arab schizophrenic patients were significantly greater in comparison with those of control group (Akanji *et al.*, 2009).

The evaluation of serum levels of CRP in individuals with schizophrenia indicated the association with the severity of cognitive impairment but not with psychiatric symptoms (Dickerson *et al.*, 2009). The reasons for the association between *CRP* and cognitive impairments are not known with certainty but are likely to be related to an inflammatory process occurring within the vasculature of the central nervous system. The specific biological mechanisms whereby inflammation leads to cognitive impairments have not been determined, C-reactive protein has also emerged as a target for CV outcomes in schizophrenia patients (Meyer *et al.*, 2009).

Despite *CRP* gene may, therefore, be considered as relevant candidate gene for susceptibility to schizophrenia or as a gene modifier of this disease symptomatology, we observed no association of investigated genetic variants within *CRP* gene and the schizophrenia. This study has some limitations because of relatively small sample size of both groups (103 patients with schizophrenia and 105 healthy subjects) and also the absence of functional data: serum CRP levels in correlation with genotypes have not been measured.

In conclusion, in this pilot study no association of selected *CRP* gene rs1417938, rs1800947 and rs1205 variants and susceptibility to schizophrenia in Armenian population was observed.

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Table 1: Distribution of *CRP* genotypes and carriage rates of *CRP* minor alleles for three investigated single nucleotide polymorphisms (SNPs) in controls and patients with schizophrenia (SCZ). The data are presented as absolute numbers with proportions in parallel.

***CRP* SNP**	**Genotype 1**	**Genotype 2**	**Genotype 3**	**Allele 1**	**Allele 2**	**Carriage 2**	**Haplotype frequency**		
rs1417938	TT	TA	AA	T	A	A	Sequence	Control	SCZ
Control	56 (0.53)	44 (0.42)	5 (0.05)	156 (0.74)	54 (0.26)	49 (0.47)	T-C-C	0.47765	0.39498
SCZ	56 (0.54)	26 (0.35)	4 (0.11)	148 (0.72)	58 (0.28)	47 (0.46)			
rs1800947	CC	CG	GG	C	G	G	A-C-C	0.254	0.28501
Control	100 (0.95)	5 (0.05)	0 (0.000)	205 (0.98)	5 (0.02)	5 (0.05)			
SCZ	96 (0.93)	7 (0.07)	0 (0.000)	199 (0.97)	7 (0.03)	7 (0.07)	T-C-T	0.24865	0.2851
rs1205	CC	CT	TT	C	T	T			
Control	58 (0.55)	38 (0.36)	9 (0.09)	154 (0.73)	56 (0.27)	47 (0.45)	T-G-T	0.0197	0.03491
SCZ	45 (0.44)	48 (0.47)	10 (0.10)	138 (0.67)	68 (0.33)	58 (0.56)			