



•Фпрбшршршиши и пъишиши приионър •Экспериментальные и теоретические статьи• • Experimental and Theoretical articles •

**Biolog. Journal of Armenia**, 1 (62), 2010

## SINGLE NUCLEOTIDE POLYMORPHISMS OF THE COMPLEMENT SYSTEM C1Q PROTEIN IN SCHIZOPHRENIA

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Schizophrenia is a complex, multifactor psychiatric disorder. Our previous findings indicated that altered functional activity of the complement system, a major mediator of the immune response, is implicated in the pathogenesis of schizophrenia. In the present study single nucleotide polymorphisms (SNPs) of the complement C1q component were investigated in schizophrenia-affected subjects. For this purpose 103 schizophrenics and 105 healthy controls were genotyped for C1q four SNPs (C1qA rs292001, C1qB rs913243, rs291982 and rs631090) using PCR-SSP and Real-time PCR methods. The data obtained indicate no association of C1qA rs292001, C1qB rs913243, rs631090 polymophisms with schizophrenia whereas the carriers of the C1qB rs291982\*T mutant allele were significantly less frequent in patients when compared to control (62% vs 78%, pnomi-nal=0.012, OR=0.456, 95%CI: 0.53-0.91, pcorrected=0.048). The results of the present study suggest the association of C1qB rs291982 polymorphism with schizophrenia.

### C1q - complement system - schizophrenia - single nucleotide polymorphisms

Շիզոֆրենիան բարդ, բազմագործոն բնույթի հոգեկան հիվանդութուն է։ Մեր նախորդ հետազոտությունների արդյունքները վկայում են, որ շիզոֆրենիան զուգակցվում է իմունային պատասխանի կարևորագույն միջնորդի՝ կոմպլեմենտի համակարգի ֆունկցիոնալ ակտիվության խախտմամբ։ Տվյալ աշխատանքում ուսումնասիրվել են կոմպլեմենտի C1q բաղադրիչը կոդավորող գենի եզակի նուկլեոտիդային պոլիմորֆիզմները (ԵՆՊ) շիզոֆրենիայով հիվանդների մոտ։ Այս նպատակով 103 շիզոֆրենիայով հիվանդների և 105 առողջ անձանց ԴՆԹ-ի նմուշները գենատիպավորվել են C1q-ի չորս ԵՆՊ-երի համար (C1qA: rs292001, C1qB: rs913243, rs291982 և rs631090)՝ կիրառելով PCR-SSP և Real-time PCR մեթոդները։ Համաձայն ստացված տվյալների՝ C1qA rs292001, C1qB: rs913243 և rs631090 ԵՆՊ-երն ասոցիացված չէին շիզոֆրենիայի հետ, մինչդեռ C1qB rs291982\*T մուտանտ ալելի կրողների քանակը զգալիորեն քիչ էր հիվանդների մոտ՝ առողջ անձանց համեմատությամբ (62% vs 78%, pnominal=0.012, OR=0.456, 95%CI: 0.53-0.91, pcorrected=0.048)։ Տվյալ հետազոտության արդյունքները վկայում են, որ C1qB rs291982 պոլիմորֆիզմը ասոցիացված է շիզոֆրենիայի հետ։

> Clq ( կոմպլեմենտի համակարգ ( շիզոֆրենիա ( եզակի նուկլեոտիդային պոլիմորֆիզմներ

Шизофрения является комплексным психическим заболеванием многофакторной природы. Результаты наших предыдущих исследований свидетельствуют о том, что шизофрения сопровождается нарушением функциональной активности важнейшего медиатора иммунного ответа – системы комплемента. В данной работе исследованы единичные нуклеотидные полиморфизмы (ЕНП) С1q компоненты комплемента у больных шизофренией. С этой целью образцы ДНК 103-х больных шизофренией и 105-ти здоровых лиц были генотипированы по четырем ЕНП С1q (С1qA: rs292001, С1qB: rs913243, rs291982 и rs631090) с помощью методов PCR-SSP и Real-time PCR. Согласно полученным данным, С1qA rs292001, С1qB: rs913243, rs631090 полиморфизмы не ассоциировали с шизофренией, тогда как носителей С1qB rs291982\*Т мутантной алели среди больных было значительно меньше, чем среди здоровых лиц (62% vs 78%, pnominal=0.012, OR=0.456, 95%CI: 0.53-0.91, pcorrected=0.048). Результаты настоящего исследования свидетельствуют о том, что С1qB rs291982 полиморфизм ассоциирует с шизофренией.

# C1q – система комплемента – шизофрения – единичные нуклеотидные полиморфизмы

Schizophrenia is a complex and severe psychiatric disorder (ICD 10 code: F20), manifested by a disruption in cognition and emotion along with negative (avolition, alogia, apathy, poor social functioning) and positive (hallucinations, delusions) symptoms [20]. According to the neurodevelopmental theory, the etiology of schizophrenia may involve pathological processes before the brain approaches its adult anatomical state. These processes are induced by environmental factors and result in both genetic and congenital abnormalities. They in their turn lead to disfunction of specific neural networks that would account for premorbid signs and symptoms observed in individuals that later develop schizophrenia [reviewed in 7]. Evidence from genetic studies suggest a high degree of heritability of schizophrenia and point to a number of potential candidate genes (Fig. 1) that may be perturbed early in development leading ultimately to the development of psychotic symptoms [reviewed in 2, 7, 10]. However, the molecular etiopathomechanisms of this disorder are still unclear.



**Fig. 1.** Schizophrenia gene linkage map [21]. Candidate genes are indicated by horizontal lines; number of candidate genes is indicated under chromosome serial number.

Recent studies performed in our laboratory indicate the crucial role of the alterations in the immune system state in schizophrenia and provide evidence on the implication of the alterations in the major mediator of the immune response, the complement system, in pathogenesis of this disorder [3, 4, 8, 12-16]. In paticular, increased levels of the complement system C1q protein, initiator of the complement classical cascade, in schizophrenia-affected subjects has been found [8, 13]. This finding is of special interest accounting for a possitive linkage of schizophrenia with chromosome 1p31 loci located nearby C1q gene (1p36.12) [9, 17]. To explore whether this alterations are genetically determined or not, in the present study we evaluated the possible association between susceptibility to schizophrenia and C1q gene variants in

Armenian population, by focusing on four single nucleotide polymorphisms (SNPs) frequent in European population. This is the first study investigating association of C1q polymorphisms with schizophrenia.

*Material and Methods.* <u>Study population.</u> In total, 208 unrelated Caucasian individuals of Armenian nationality living in Armenia (103 schizophrenic patients and 105 healthy individuals) were enrolled in this study. All patients (mean age±SD was  $46\pm9.88$  years) were diagnosed as paranoid schizophrenics (F20.0) according to the ICD-10 criteria [20] by two independent experienced psychiatrists. The affected subjects were recruited from the Nubarashen and Nork Clinics of Psychiatric Medical Center MH RA. Healthy volunteers (mean age±SD was  $37\pm11.32$ ) without family history of schizophrenia, recruited from the Erebouni Medical Center MH RA, served as reference control population samples. All individuals gave their informed consents to provide 5 ml of venous blood. This study was approved by the Ethical Committee of the Institute of Molecular Biology NAS RA.

<u>Methods. Genomic DNA extraction.</u> Genomic DNA samples were isolated from fresh blood according to the standard phenol-chloroform method [18] and stored at  $-30^{\circ}$ C until further use.

Genotyping analysis. All DNA samples were genotyped for C1q four SNPs, namely C1qA rs292001, ClqB: rs913243, rs291982 and rs631090. ClqA rs292001, ClqB rs291982 and rs631090 SNPs were screened by polymerase chain reaction with sequence-specific primers (PCR-SSP) under the conditions described elsewhere [5]. All primers for PCR-SSP were designed using the genomic sequences in the GenBank (http://www.ncbi.nlm.nih.gov, GeneID:712, 713). The primer sequences for three SNPs were: 1) rs292001: allele G, reverse 5'GAT GCC CGG ATG CAA ATT AC, allele A, reverse 5'GAT GCC CGG ATG CAA ATT AT, constant forward 5'AGG CTT CAG AGA CTC ACA TTC; 2) rs291982: allele G, reverse 5'ACC TTT GCC CAG ATC CAA ATG, allele T, reverse 5'ACC TTT GCC CAG ATC CAA ATT, constant forward 5'AGC CAC AAG TCC CAA TGA GA; 3) rs631090: allele T, forward 5'CAC GGA TCT CTT ACC ATT AAA T, allele C, forward 5'CAC GGA TCT CTT ACC ATT AAA C, constant reverse 5'CAT CTG TGA AAT GGG GAT GAA. The presence/absence of allele-specific amplicons in the genotyping products was visualized by 2% agarose gel stained with ethidium bromide fluorescence in reference to a molecular weight marker. Genotypes for C1qB rs913243G/T SNP were determined using TaqMan SNP genotyping assay (Applied Biosystems, Assay ID C 3176751 10) according to the manufacturer's instructions. Randomly selected samples (n=21; 10% of total) were amplified twice to check for confidence of genotyping, and in each case complete concordance was obtained.

**Data analysis.** The distributions of genotypes for all investigated SNPs were checked for correspondence to the Hardy-Weinberg (H-W) equilibrium. To find potential relevance of targeted *C1q* SNPs to schizophrenia, their allelic (gene) and phenotype frequencies (carriage rates) in patients and control groups were compared. The calculations of allelic and phenotype frequencies were based on the observed number of genotypes. Haplotype analysis and the extent of genetic association or linkage disequilibrium (LD) between different loci on specific chromosome were estimated as described earlier [22]. Maximum-likehood (ML) haplotype frequencies in patients and control groups were estimated using an expectation-maximization (EM) algorithm [6]. The significance of differences between allele and phenotype frequencies in both groups was determined using Pearson's Chi-square test. The odds ratio (OR), 95% confidence interval (CI), and Pearson's p-value were calculated. Statistical power of the present study was calculated according to the protocol described elsewhere [11]. P values were adjusted by Bonferroni multiple correction approach, and those less than 0.05 were considered statistically significant. Data analysis was performed by the use of SNP analyzer [22] and SPSS (SPSS Inc, USA) softwares.

**Results and Discussion.** To determine whether four selected polymorphisms in the C1q gene are associated with schizophrenia, the DNA samples of patients with schizophrenia and ethnically matched individuals from control group were genotyped. The genotype distributions of studied SNPs in *both patients and control groups were in Hardy-Weinberg equilibrium.* Statistical power of the present study, indicating the differences in the carriage of the C1q rs291982\*T allele between the patients and healthy controls for the odds ratios (OR) 1.5 and 2, was 93.7% and 56.1%, respectively.

SNP ID	Genotype 1	Genotype 2	Genotype 3	Allele 1	Allele 2	Carriage
rs292001	GG	GA	AA	G	А	А
SCZ	36 (0.35)	57 (0.55)	10 (0.10)	129 (0.63)	77 (0.37)	65 (0.65)
Control	26 (0.25)	61 (0.58)	18 (0.17)	113 (0.54)	97 (0.46)	79 (0.75)
p-value			0.116 <sup>a</sup>		0.069 <sup>b</sup>	0.108 <sup>c</sup>
rs913243	GG	GT	TT	G	Т	Т
SCZ	32 (0.31)	52 (0.50)	19 (0.18)	116 (0.56)	90 (0.44)	71 (0.69)
Control	30 (0.30)	57 (0.57)	13 (0.13)	125 (0.60)	85 (0.40)	72 (0.69)
p-value			0.282 <sup>a</sup>		0.510 <sup>b</sup>	0.955°
rs291982	GG	GT	TT	G	Т	Т
SCZ	39 (0.38)	42 (0.41)	22 (0.21)	120 (0.58)	86 (0.42)	64 (0.62)
Control	23 (0.22)	57 (0.54)	25 (0.24)	103 (0.49)	107 (0.51)	82 (0.78)
p-value			0.673 <sup>a</sup>		0.059 <sup>b</sup>	0.012 <sup>c</sup>
rs631090	TT	TC	CC	Т	С	C
SCZ	69 (0.67)	31 (0.30)	3 (0.03)	169 (0.82)	37 (0.18)	34 (0.33)
Control	78 (0.74)	25 (0.24)	2 (0.02)	181 (0.86)	29 (0.14)	27 (0.26)
p-value			0.641 <sup>a</sup>		0.246 <sup>b</sup>	0.248 <sup>c</sup>

**Table 1.** Distribution of C1q genotypes and carriage rates of C1q minor alleles for four investigated SNPs in patients with schizophrenia (SCZ) and healthy controls.

The data are presented as absolute numbers along with proportions. a) p-values for comparison of Allele 2 carriage (phenotype frequency) between SCZ patients and control subjects; b) p-values for comparison of Allele 2 proportion (allelic frequency) between SCZ patients and control subjects; c) p-values for comparison of Genotype 3 proportion (versus other genotypes) between SCZ patients and control subjects.

The frequencies of all studied C1q variants in schizophrenia patients and control subjects are shown in Table 1. C1qA rs292001, C1qB rs913243, rs631090 variants were equally represented both in schizophrenics and controls. By contrast, the carriers of the C1qB rs291982\*T mutant were significantly less frequent in patients with schizophrenia when compared with control groups (62% vs 78%, pnominal=0.012, OR=0.456, 95%CI: 0.53-0.91).



**Fig. 2.** Proportion (%) of C1qB rs291982\*T mutant allele carriers (phenotype frequency) in patients with schizophrenia and healthy controls. Pnominal=0.012 (pcorrected<0.05), OR=0.46, 95% CI= 0.53-0.91.

In Fig. 2 the carriers of C1qB rs291982\*T mutant allele are represented. The p-value for comparison of mutant allele carriers remained significant after Bonferonni correction for the number of loci (pcorrected=0.048). P-value for comparison of C1qB rs291982\*T mutant allele frequencies was moderately significant (41% in patients vs 51% in controls, pnominal=0.059) only before Bonferonni correction (pcorrected=0.24). These results suggest that the C1qB rs291982\*T mutant allele might be protective for schizophrenia patients. In other words, the wild rs291982G allele might be considered as a risk factor for schizophrenia at least in Armenian population.

To confirm the association revealed and to estimate the ML haplotype frequencies, the haplotype analysis was performed. In total, 13 haplotypes in each group of study subjects were estimated. Interestingly, haplotype comparison between patients and healthy controls revealed four haplotypes, namely, G-T-G-T (pnominal=0.004, pcorrected=0.056), A-G-T-T (pnominal=0.005, pcorrected=0.07), G-G-G-C (pnominal=0.009, pcorrected=0.13) and A-T-T-T (pnominal=0.048, pcorrected=0.67), only one of which (G-T-G-T) remained moderately significant after the correction for the number of haplotypes (in total, 13 haplotypes). The SNP comparison analysis confirmed association of C1qB rs291982 variant with schizophrenia detected in this study.

There are limited data on functionality of the C1qB rs291982 polymorphism. In order to explore possible functional role of this genetic variant, the web server FASTSNP [23] was used for identification of the functional effects of SNPs. C1qB rs291982 SNP is an intronic enhancer (alters a binding site of a transcription factor in an intronic region). The C1qB rs291982 wild G allele contains binding site of HFH-2 transcription factor, which is absent in the mutant allele. While these types of functional effects have low phenotypic risk [19], our finding might indicate the functionality of C1qB rs291982 SNP since the enhancer is transcription-stimulating DNA regulatory element [1].

Finally, the present study is the first investigation concerning the association of C1q genetic variants with schizophrenia, and reveals the negative association of C1qB rs291982 SNP with susceptibility to schizophrenia in Armenian population. In addition, the results of this study provide evidence that alterations in the complement classical pathway in schizophrenia are genetically determined.

### Acknowledgements

The author express her gratitute to the administration and medical staff of Psychiatric Medical Center (Nork and Nubarashen clinics) and Erebouni Medical Center MH RA, performing diagnostics of the patients and examination of healthy individuals involved in the study, and providing blood samples of these subjects; to the head and scientific staff of the Laboratory of Immunogenomics and Proteomics of the Medical Faculty of Palacky University (Chech Republic) and to the scientific staff of the Laboratory of Macromolecular Complexes of the Institute of Molecular Biology NAS RA for technical support, assistance and help provided during the implementation of this study.

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*Received* 25.02.2010.