••• ГЕНЕТИКА ••• GENETICS •••

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Structure of Ukrainian population on SNP *rs3093059* of C-reactive protein gene

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The distribution of single nucleotide polymorphism (SNP) *rs3093059* of the C-reactive protein gene in 95 persons – Russians and Ukrainians – residents of Kharkov city have been investigated. One-nucleotide replacement *757C/T*, which results in a change of the amino-acid sequence of C-reactive protein, was performed using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP). Major allele in the studied population is *T* (p=0.89). Homozygous *TT* genotype was found in about 78%, heterozygous *TC* in 22% of people. Individuals with genotype *CC* in the sample were not detected. We have tested the sample for compliance with Hardy-Weinberg equilibrium. The calculations showed that the distribution of genotypes in the studied sample were not significantly different from the theoretically expected. The frequencies of genotypes and alleles calculated on the data of our investigation can serve as a starting point for studies on the search for markers of genetic susceptibility to diseases under the control of the studied gene.

Key words: *C-reactive protein, single nucleotide polymorphism, Hardy-Weinberg equilibrium.*

Структура украинской популяции по SNP *rs3093059* гена С-реактивного белка

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Изучено распределение однонуклеотидного полиморфизма (SNP) *rs3093059* гена С-реактивного белка у 95 жителей Харькова – русских и украинцев. Однонуклеотидная замена *757C/T*, приводящая к изменению аминокислотной последовательности в С-реактивном белке, была изучена с помощью полимеразной цепной реакции с использованием эндонуклеазы *TasI*. В изученной популяции мажорным аллелем является *T* (p=0,89). Гомозиготный *TT* генотип был обнаружен у 78%, гетерозиготный *TC* у 22%. Индивиды с генотипом *CC* в выборке не были обнаружены. Распределение генотипов значимо не отличается от теоретически ожидаемого равновесного состояния. Рассчитанные частоты генотипов и аллелей могут служить отправной точкой для расчёта риска предрасположенности к заболеваниям, которые находятся под контролем данного гена.

Ключевые слова: *С-реактивный белок, однонуклеотидный полиморфизм, равновесие Харди-Вайнберга.*

Структура української популяції за SNP *rs3093059* гену С-реактивного білка

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Вивчено розподіл однонуклеотидного поліморфізму (SNP) *rs3093059* гена С-реактивного білка у 95 мешканців Харкова – росіян і українців. Однонуклеотидна заміна *757C/T*, що призводить до зміни амінокислотної послідовності в С-реактивному білку, була вивчена за допомогою полімеразної ланцюгової реакції з використанням ендонуклеази *TasI*. У популяції, що вивчена, мажорним алелем є *T* (p=0,89). Гомозиготний *TT* генотип був знайдений у 78%, гетерозиготний *TC* у 22% випадків. Індивіди з генотипом *CC* у виборці не були знайдені. Розподіл генотипів значущо не відрізнявся від теоретично очікуваного рівноважного стану. Розраховані частоти генотипів і алелей можуть слугувати відправною точкою для розрахунку ризику схильності до захворювань, що знаходяться під контролем даного гена.

Ключові слова: *С-реактивний білок, однонуклеотидний поліморфізм, рівновага Харди-Вайнберга.*

Introduction

The acute phase protein C-reactive protein (CRP) can be assessed as a marker of inflammation. More recently, CRP has not been only to be considered a marker, but also a potential participant in the pathogenesis of cardiovascular disease, and various roles in cellular activation and in inflammatory processes have been proposed (Pepys, Hirschfield, 2003). Very recently, it has become firmly established that a genetic component exists for CRP. Baseline levels of CRP show a clear heritability of 40% (Pankow et al., 2001) and 39% (Vickers et al., 2002) in family studies. CRP heritability is approximately 0.3–0.4 in multiple populations, including Caucasian Americans, Caucasian Europeans, Japanese Americans, and Native Americans (Lange et al., 2006a). As CRP plays a substantial role in acute-phase inflammation responses and interacts with a variety of cytokines and immune cells (Elliott et al., 2009; Ridker et al., 2008), it would be of great interest whether the genetic loci associated with CRP levels are associated with hematological or biochemical traits (Goldman et al., 1987; Carlson et al., 2005). C-reactive protein gene was subsequently mapped to the proximal long arm of chromosome 1 in the 1q23.2 region (Floyd-Smith et al., 1986; Walsh et al., 1996), it is composed of 1 intron separating 2 exons. The first exon encodes a signal peptide and the first 2 amino acids of the mature protein. This is followed by a 278-nucleotide-long intron that includes a GT repeat sequence. The second exon encodes the remaining 204 amino acids, followed by a stop codon (Goldman et al., 1987). Several population-based association studies have shown that common genetic variants at the CRP locus are significantly associated with plasma CRP levels (Miller et al., 2005; Brull et al., 2003). The study of population structure polymorphisms of this gene is of practical importance, since it may serve as the basis of studies similar to the distribution of polymorphisms in patients with cardiovascular, endocrine and other diseases. This may lead to disturbances in metabolic pathways that are controlled by this gene. We aimed to investigate the distribution of single nucleotide polymorphism of the C-reactive protein gene in the Slavonic population (Ukrainian and Russian) from Kharkov city.

Materials and methods

DNA of 95 persons – Russians and Ukrainians – residents of Kharkov city have been investigated with the written agreement of people. DNA was separated from leucocytes with a help of ion-exchange gum Chelex-100 (Walsh et al., 1991). One-nucleotide replacement *757C/T*, which results in a change of the amino-acid sequence of C-reactive protein was determined by amplification in the polymerase's chain reaction using *TasI* endonuclease. We used forward (*GCTATGTCTGTGATCAGGCA*) and reverse (*CCAAACACCGCATGTTCTC*) primers. DNA *pUC19* was hydrolyzed with endonuclease *MspI*. The DNA fragments were separated after restriction with the help of electrophoresis in 2% agarose gel. The electrophoregram of PCR-products (Fig. 1) provides insight into genotypes of donors of the *CRP* gene. One band which corresponds to DNA fragment 148 bp (was not observed) indicates a *CC* genotype. In sample 1–3, 5, 7–12, 14, 15, 19, 20 (genotype *TT*) two bands present DNA fragments with lengths 97 and 51 bp. Three bands (148, 97 and 51 bp) in samples 4, 6, 13, 16–18 indicate the *CT* genotype. When comparing observed and expected frequencies of genotypes the χ^2 test with Yates correction was used. To calculate the statistical errors of frequencies and fractions, their confidence intervals were calculated using φ-transformation and the criteria *F* (Armitage, Berry, 1994). The checkup of statistic hypotheses about the association of the studied alleles was conducted with a help of χ^2 criteria in the significance level $p \leq 0.05$.

Results and discussion

Major allele in the studied population is *T* (p=0.89), minor allele frequency of *C* is equal to 0.11. Homozygous *TT* genotype was found in about 78%, heterozygous *TC* in 22% of people. Individuals with genotype *CC* in the sample were not detected (Table 1). The absence of a particular genotype in the sample may be due to the effect of sampling – a rare genotype is less likely to be discovered. Another reason may be the selective importance of polymorphism, by which a given genotype may be absent in living humans. To clarify this assumption we have tested the sample for compliance with Hardy-Weinberg equilibrium. The calculations showed that the distribution of genotypes in the studied sample were not significantly different from the theoretically expected (Table 1). If the polymorphism is selectively neutral, in the studied population about 1% of genotype *CC* should exist. There is a small opportunity to find rare genotype in rather small sample.

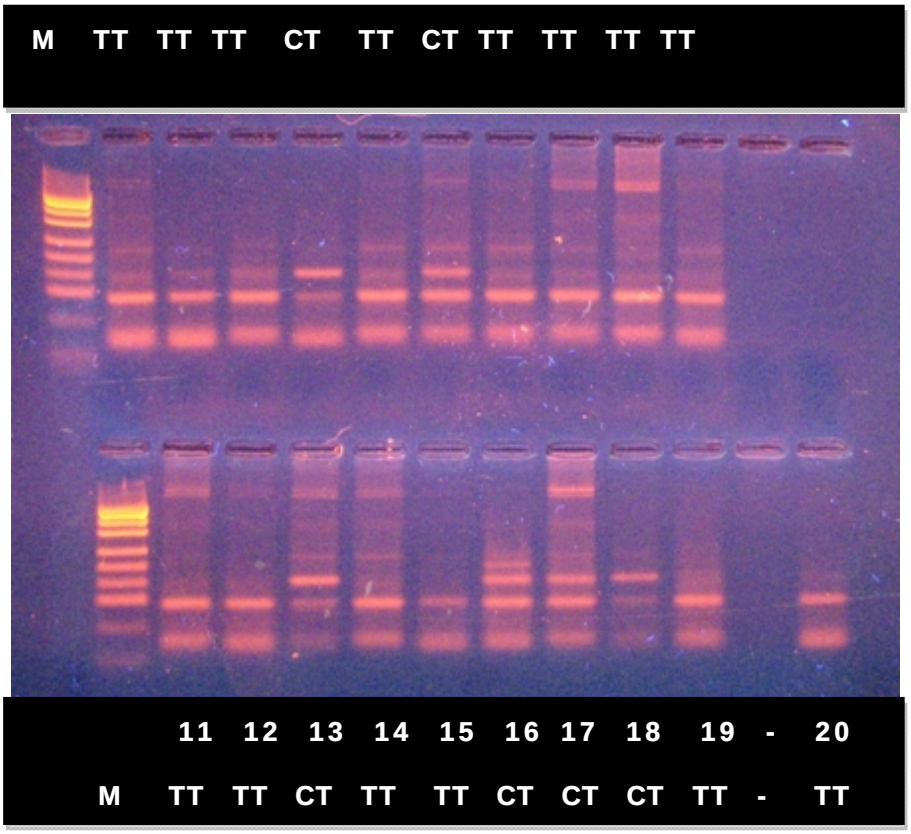

Fig. 1. Electrophoregram of PCR product of specific sequence of DNA genotyped on SNP polymorphism of *CRP* gene (M – DNA marker; *TT*, *CT* – genotypes)

But we mustn't throw away an idea of selective importance of polymorphism under study. Table 2 shows the frequencies of minor alleles *C* in different populations. The maximum frequency of this allele is present in the Caucasian American (0.938) and in the Northern European descent (0.92). The rarest allele that is observed in Indo-European (0.12), but this allele was not detected in Caucasian European. We should pay attention that in some populations this polymorphism is associated with type 2 diabetes and cardiovascular diseases (Table 3). Our sample data indicate that in patients with type 2 diabetes the frequency of minor allele is higher than among healthy people although the difference was not statistically significant. The frequencies of genotypes and alleles calculated on the data of our investigation can serve as a starting point for studies on the search for markers of genetic susceptibility to diseases under the control of the studied gene.

Table 1.

The distribution of genotypes of SNP *rs3093059 CRP* gene in Kharkov population

Statistical parameter		Genotype			Allele	
		TT	*TC*	*CC*	*T*	*C*
Quantity, n	95	74	21	0	0.89	0.11
Quantity, %	100.0	77.9	22.1	0.0		
95% CI,		68,8-85,8	14,3–31,1	0,0–1,1	0.84–0.93	0.07–0.16
Fraction at HWE	1.000	0.792	0.196	0.012		
Quantity at HWE, n	95	75.3	18.6	1.1		
Statistics	χ^2=0.43; $\chi^2_{(0.05)}$=3.8; p>0.05					

Remark: HWE – Hurdy-Weinberg equilibrium, CI – confidence interval.

Table 2.

Allele frequencies of SNP *rs3093059 CRP* in different populations

Population	*C* allele frequency	HWE (Yes/No)	Reference
Northern European descent	*CC*=0.92	Yes	Kim et al., 2008
Indo-European	0.12	Yes	Mahajan et al., 2011
Caucasian American	0.938	Yes	Qingwei et al., 2006
Caucasian European	0.00	No	Shih, 2007
Caucasian American	0.075	Yes	Perry et al., 2009

Remark: HWE – Hurdy-Weinberg equilibrium.

Table 3.

Association of different type of *CRP* gene polymorphism in different populations

Population	Polymorphism	Association with disease	Reference
Non Hispanic black	Rs2808630 AG	Chronic kidney disease	Hung et al., 2011
Native American	rs1800947	Coronary heart disease	Pai et al., 2008
European American	rs1417938	Stroke	Lange et al., 2006b
African American	rs3093058	Myoinfarction	Lange et al., 2006b
Austrian	rs1417938	Cervical cancer	Polterauer et al., 2011
Pima India	rs 133552	Diabetes mellitus	Wolford et al., 2003

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