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ARCTIC MEDICINE

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ASSOCIATION OF GENE POLYMORPHISM *PTGS2* rs689466 WITH PLASMA IRISIN LEVEL IN YAKUTS

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Prostaglandin E2 may be involved in an increase in body temperature during cold stress by the type of fever. At the same time, a significant part of heat production is produced during shivering thermogenesis, due to arbitrary muscle activity, which is accompanied by the release of the hormone irisin. Prostaglandin E2 is formed from arachidonic acid by the enzyme cyclooxygenase-2, which is encoded by the *PTGS2* gene. The transcriptional activity of the *PTGS2* gene depends on its allelic variants, which can affect thermoregulation processes in different ways. In this regard, the aim of this study is to analyze the polymorphism rs689466 of the *PTGS2* gene with the level of irisin in blood plasma in a population of Yakuts living in cold climatic conditions. The study involved 183 women and 80 men (average age 19.73±1.99 years). Analysis of the association of polymorphism rs689466 of the *PTGS2* gene with irisin levels showed that in men with the TT genotype, irisin levels (8.2±1.85 μg/ml) were statistically significantly higher (U=261; p=0.005), compared with men with CT+CC genotypes (7.1±1.25 μg/ml). In addition, it was found that men with the TT genotype (63.6±6.67 kg) had a lower weight than men with the CT+CC genotypes (67.93±7.28 kg; U=279; p=0.01). The detected association of the TT rs689466 genotype of the *PTGS2* gene with elevated irisin levels and with a lower weight in men may indicate the effect of prostaglandin E2 on shivering thermogenesis during cold stress, which may play a role in human adaptation to a cold climate.

Keywords: irisin, prostaglandin E2, gene *PTGS2*, rs689466, fever, cold stress.

Introduction. One of the key mechanisms in human physiology is the ability to sense and regulate body temperature, which is crucial for survival. The body's defense reactions include fever, which is accompanied by an increase in body temperature in response to pyrogens [14] that stimulates an immune response [6]. An increase in body temperature during fever occurs due to shivering (in skeletal muscles) and nonshivering (in brown adipose tissue) thermogenesis, and a decrease in passive heat loss occurs due to vasoconstriction [8]. However, the main contribution to increased heat production in fever is made by shivering thermogenesis, which is accompanied by involuntary muscle contraction (shiver) and the release of the hormone irisin into the blood [7,15].

Prostaglandin E2 is a principal fever mediator that can also control the basal mechanisms of thermoregulation. In 2015, J. Foster and his colleagues pub-

lished a paper on a new hypothesis about the role of prostaglandin E2 in thermoregulation processes under cold stress [12]. The main propositions of the hypothesis are based on the fact that cold-sensitive neurons and EP3 neurons (prostaglandin E2 receptor) activate the same areas of the hypothalamus that are responsible for thermoregulation [12]. In this regard, involuntary thermogenic reactions to maintain body temperature during cold stress are identical to the mechanisms that increase body temperature during fever [12].

Prostaglandin E2 is formed as a result of oxygenation and cyclization of arachidonic acid by the enzyme cyclooxygenase-2 [1]. The cyclooxygenase-2 enzyme is encoded by the *PTGS2* gene [2] located on chromosomal region 1q25.2-q25.3 and containing 10 coding exons [2,3]. A large number of single-nucleotide polymorphic regions (SNPs) are known in the *PTGS2* gene, some of which are considered functionally significant [13]. These regions include SNP rs689466, which is located in the promoter region of the *PTGS2* gene [10, 13]. Analysis of mRNA in human esophageal tissues showed that the normal T allele rs689466 leads to a higher transcriptional activity of the *PTGS2* gene compared to the mutant C allele [4, 10]. Since there is variability in the transcriptional activity of *PTGS2* depending on allelic variants of the rs689466 polymorphism [4, 10], it is likely that this may affect the role of prostaglandin E2 in thermoregulation under cold stress.

In this regard, the aim of this work is to analyze the relationship of the rs689466 polymorphism of the *PTGS2* gene with the irisin level in blood plasma in Yakuts living in cold climatic conditions.

Material and Methods. *Subjects.* The study involved 263 Yakuts (183 women and 80 men), with an average age of 19.73±1.99 years. At the time of the study, none of the participants had any health complaints. The study participants filled out a questionnaire on their own, indicating their gender, ethnicity, and age. All participants gave written informed consent to participate in the study. Study was approved by the local Biomedical Ethics Committee at the Yakut Scientific Center of Complex Medical Problems, Siberian Branch of the Russian Academy Scientific of Medical Sciences, Yakutsk, Russia (Yakutsk, Protocol No. 16, and 13 December 2014).

Anthropometric parameters. Anthropometric parameters (body weight in kilograms, height in centimeters) were measured in all participants using standardized methods. Body mass index (BMI) was calculated by dividing body mass by the square of height. The sample was divided into three groups according to BMI categories [11]: underweight (≤18.49 kg/m^2), normal weight (18.5–24.99 kg/m^2), and overweight/obese (≥25 kg/m^2).

ELISA of irisin levels. Irisin levels in fasting blood plasma (μg/ml) were determined using an enzyme-linked immunosorbent assay (ELISA) "Irisin ELISA BioVendor" (BioVendor-Laboratorni medicina A..S., Czech Republic). Irisin concentration in the samples was measured at a wavelength of 450 nm on a VICTOR X5 Multilabel Plate Reader Reader (Perkin Elmer Inc., USA).

PCR-RFLP analysis of rs689466 of the PTGS2 gene. Genomic DNA was isolated from blood by phenol-chloroform extraction. Genotyping was performed using PCR-RFLP analysis. The original oligonucleotide primers were selected using the FastPCR program (http://primerdigital.com/). The following primer sequences were used for rs689466 of the *PTGS2* gene: F: 5'-ATGAGTTGTGACCATGGATCAA-3', R: 5'-AAAAACCTCCAAGTGAGTCTCTT-3'. Detection was performed using standard PCR on a T100 Thermal Cycler (Bio-Rad, Hercules, USA). The PCR conditions for rs689466 were as follows: denaturation-95°C (5 min), annealing-58°C (45 sec), elongation-72°C (7 min), a total of 30 cycles. Restriction fragment length polymorphism (RFLP) analysis was performed using endonuclease *Bst4C* I (SibEnzyme, Russia), in accordance with the manufacturer's recommendations. After incubation with *Bst4C* I, the T allele of rs689466 remains intact (432 bp), while the C allele is split into 295 bp and 137 bp. The hydrolysis products were separated in horizontal electrophoretic chambers in 2% agarose gel. Electrophoregrams were visualized using gel-video documentation systems from Bio-Rad (Hercules, USA).

Statistical analysis. The data obtained were analyzed using the statistical program Statistica 13.5 (TIBCO Software Inc., USA). Quantitative results are presented as "mean ± standard deviation". The frequency of genotypes of the rs689466 polymorphism of the *PTGS2* gene in the Yakut population (n=263) was checked for compliance with the Hardy-Weinberg equilibrium using the χ^2 criterion. To check the normality of the distribution, the Kolmogorov-Smirnov test was performed. Associations between the rs689466 genotypes of the *PTGS2* gene

Table 1

Average irisin levels (μg/ml) in men and women, taking into account BMI

BMI categories	Women	Men
Underweight	7.88±1.96 (n = 25)	8.52±2.64 (n = 11)
Normal weight	8.43±2.94 (n = 142)	7.65±1.66 (n = 60)
Overweight	8.27±1.96 (n = 16)	9.17±2.11 (n = 9)

Table 2

Associative analysis of irisin levels and anthropometric parameters with rs689466 genotypes of *PTGS2* gene in Yakut population

Parameters	Mean ± standard deviation		*U*	*p*
	TT (n=114)	CT+CC (n=88)		
Irisin, μg/ml				
W	8.47±3.05 (n=84)	8.38±2.8 (n=58)	2397	0.87
M	**8.2±1.85** (n=30)	**7.1±1.25** (n=30)	**261**	**0.005**
Weight, kg				
W	55.87±6.28 (n=84)	55.1±5.10 (n=58)	2345	0.71
M	**63.6±6.67** (n=30)	**67.93±7.28** (n=30)	**279**	**0.01**
High, cm				
W	160.76±6.3 (n=84)	161.26±5.7 (n=58)	2332	0.67
M	172.17±5.84 (n=30)	174.37±5.71 (n=30)	382	0.32
BMI, kg/m^2				
W	21.59±1.71 (n=84)	21.17±1.48 (n=58)	2086	0.15
M	21.44±1.86 (n=30)	22.31±1.86 (n=30)	331	0.08

Note: U – the Mann-Whitney criterion; p – level of statistical significance; W – women; M – men; statistically significant differences are highlighted in bold (p<0.05)

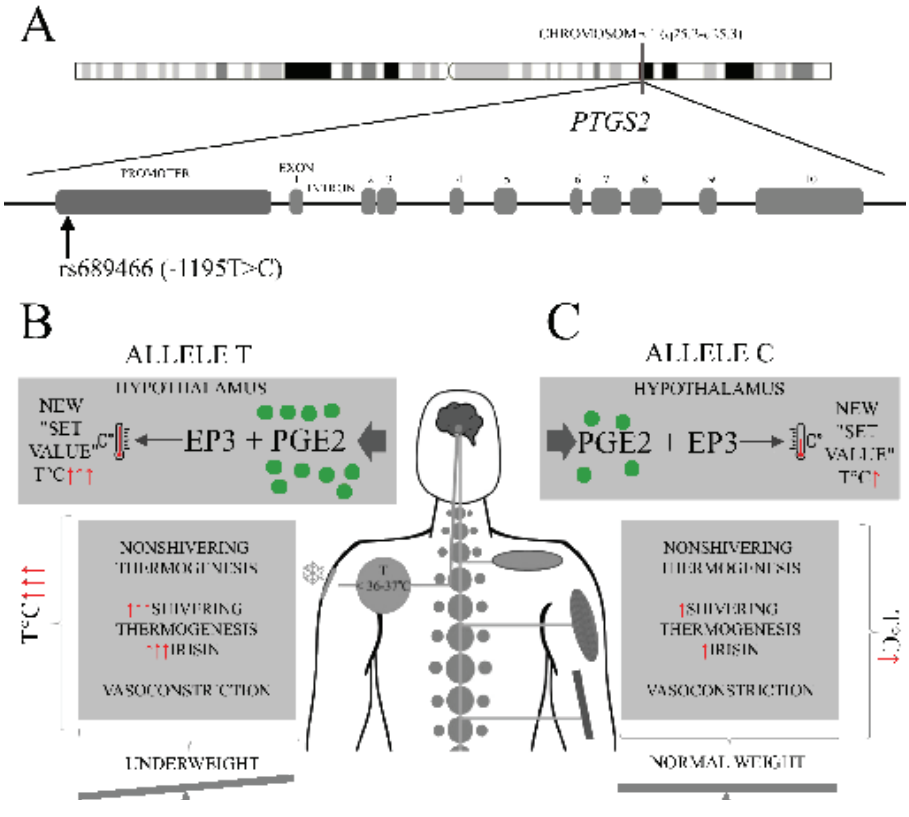

Figure. Prostaglandin E2 (PGE2) in thermoregulation mechanisms during cold stress. **A** – Localization of the *PTGS2* gene on chromosome 1 (q25.2-q25.3) and the structure of the gene indicating the location of rs689466 in the promoter region [Anyona et al., 2020]. **B** – the mechanism of PGE2 thermoregulation and its effect in carriers of the T allele rs689466 of the *PTGS2* gene. **C** – the mechanism of thermoregulation of PGE2 and its effect in carriers of the C allele rs689466 of the *PTGS2* gene.
Note: T°C is body temperature, EP3 is prostaglandin E2 receptor, ↑ is a slight increase, ↑↑↑ is a strong increase ❄ is the effect of cold on the body.

and irisin levels, weight, height, and BMI were analyzed using the Mann-Whitney *U*-test. The values of $p<0.05$ were considered statistically significant.

Results and Discussion. *Frequency distribution of alleles and genotypes of the rs689466 polymorphism of the PTGS2 gene.*

To search for a possible role of rs689466 allelic variants of the *PTGS2* gene in thermoregulation under cold stress, associative analysis of rs689466 genotypes with irisin levels in blood plasma was performed in Yakutia residents living in extremely cold climatic conditions of Eastern Siberia. The frequencies of alleles and genotypes of the rs689466 polymorphism of the *PTGS2* gene were determined in the Yakut population (n=263). The frequency of the normal T allele was 75%, and the frequency of the mutant C allele was 25%. The frequency of occurrence of the TT genotype was 55%, the heterozygous CT variant was 40%, and the CC genotype occurred with a frequency of 5%. The frequency distribution of rs689466 genotypes in the Yakut sample (n=263) corresponded to the Hardy-Weinberg equilibrium ($\chi^2=1.366$, $p=0.24$).

Irisin level depending on the genotypes rs689466 of the PTGS2 gene. Mean irisin plasma levels in women (n=183) and men (n=80), taking into account BMI, are presented in Table 1. For an associative analysis of irisin levels with rs689466 genotypes, the rare CC genotype was combined with the heterozygous CT genotype (CT+CC). The analysis was performed separately for men (n=60) and women (n=142) of normal weight. The Kolmogorov-Smirnov test revealed that irisin levels in Yakuts with normal weight (n=202) did not meet the criteria for normal distribution ($D=0.122$; $p<0.01$), so the association analysis was performed using the nonparametric Mann-Whitney *U*-test. As a result, significant associations were found in men, but not in women (Table 2). In carriers of the TT genotype, irisin levels (8.2±1.85 μg/ml) were statistically significantly higher ($U=261$, $p=0.005$) compared to the CT+CC genotypes (7.1±1.25 μg/ml) (Table 2). Additional analysis of the association of rs689466 genotypes with anthropometric parameters (weight, height, and BMI) (Table 2) showed that men with the TT genotype (63.6±6.67 kg) had a lower weight than men with the CT+CC genotypes (67.93±7.28 kg; $U=279$, $p=0.01$).

Possible mechanism of action of prostaglandin E2 in cold stress. Under conditions of thermoneutrality, for optimal life activity, the body temperature is kept in the range of 36-37°C [9]. Cold stress leads to a decrease in body temperature (<36-37°C), which in turn stimulates the synthesis of prostaglandin E2 [12]. To protect the body from hypothermia, prostaglandin E2 acts on the EP3 receptor in the preoptic region of the hypothalamus, which leads to the activation of emergency thermoregulation mechanisms similar to a fever [12].

Most likely, the higher the level of prostaglandin E2, the higher the new "set value" of body temperature will be. Since the T allele rs689466 of the *PTGS2* gene is characterized by higher transcriptional activity [4,10], we assume that carriers of the TT genotype should have higher levels of prostaglandin E2, which should lead to a higher "set value" of body temperature. As a result, carriers of the TT genotype will have a more intense or longer stage of shivering thermogenesis and, consequently, increased irisin levels in the blood than those with the CT and CC genotypes (Figure).

However, with constant exposure to cold, as in Yakutia, where winter lasts about 6 months, and the temperature of the atmospheric air during this period varies from -60°C to -20°C, the mechanism of thermoregulation by the type of febrile reaction can greatly deplete the body. Therefore, we assume that the relatively low weight of carriers of the TT genotype, compared with carriers of the CC and CT genotypes, is due to the fact that macronutrients coming from food are consumed for more intensive or prolonged shivering thermogenesis, and not for the storage function, since in cold climatic conditions the body is primarily aimed at maintaining thermal homeostasis. In turn, the adaptive role of the allele variant T rs689466 of the *PTGS2* gene is probably associated with protective mechanisms directed against extremely low atmospheric temperatures, to prevent rapid hypothermia and cold injury.

Conclusion. In the present study, the TT rs689466 genotype of the *PTGS2* gene was found to be associated with an increased irisin level and with a reduced weight in men, which may indicate the

effect of prostaglandin E2 on shivering thermogenesis under cold stress. We suggest that the increased transcriptional activity of the *PTGS2* gene in the rs689466 T allele may play a role in human adaptation to cold climates.

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SCIENTIFIC REVIEWS AND LECTURES

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UNINTENTIONAL INTRAOPERATIVE HYPOTHERMIA IN ONCOLOGICAL SURGERY AND MAINTAINING NORMOTHERAPY AS PREVENTION OF CARDIAC COMPLICATIONS: THE CURRENT STATE OF THE ISSUE

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A review is done of the latest research on unintentional intraoperative hypothermia (UIH) in oncological surgery, as well as its contribution to cardiac complications. Data are presented on the risk factors for developing UIH, the impact of surgery duration and type, as well as different anesthesia types and methods on the stage of the patient's hypothermia. Data on the relationship between the severity of UIH and the surgical profile of a patient and the patient's comorbidity were studied. It was revealed that cancer patients are at risk of developing UIH in the perioperative period, indicating the importance of preventing hypothermia during the surgery and anesthesia. The results of research on the undesirable effects of UIH, the impact of hypothermia on the development of various events, including cardiac complications, were analyzed. Data on the prevention of UIH and the methods of its prevention were systematized. The potential of maintaining the patient's normothermia for reducing the risk of developing cardiac complications in the immediate postoperative period is shown.

Keywords: unintentional intraoperative hypothermia, cardiac complications, temperature monitoring, active warming, early postoperative period.

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Unintentional intraoperative hypothermia (UIH) is a decrease in core temperature (CT) of the patient's body below 36°C during surgery. UIH is caused by adverse factors of surgical treatment that increase body heat loss (the operating room temperature, immobility of the patient, opening of body cavities and their