

Influence of Polymorphisms -824 A/G Gene of Tumor Necrosis Factor Alpha on the Basic Economic Useful Traits of Cattle

Nikolai Nikolaevich Kochnev¹, Tatiana Igorevna Krytsyna, Anastasia¹, Maksimovna Smirnova¹ and Nikolay Seraphimovich Yudin²

¹Novosibirsk State Agrarian University, Russia, 630039, Novosibirsk, Dobroljubova Street, 160

²Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Russia, 630090, Novosibirsk, Prospekt Lavrentyeva, 10.

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Tumor necrosis factor alpha gene (TNF- α) codes protein which is a multifunctional cytokine and, along with the functions of the immune response, is involved in the morphogenesis and development of the fetus, plays an important role in the formation of mammary gland, stimulates the formation of prostaglandins and regulates appetite and lipid metabolism. The aim of the work is to study the genetic structure of herds of red steppe breed by polymorphism -824 A / G gene in the promoter of the TNF- α gene, and to study possible associations of polymorphism with the main features of milk production, reproduction, growth and development of red steppe breed cattle. The sample of 148 animals the frequency of genotypes G/G, G/A and A /A, respectively, were 56%, 38% and 6% at a frequency of allele G - 75% and the allele A - 25%. Genotype frequencies distribution corresponded to Hardy-Weinberg equilibrium. Animals with genotype G / A significantly excelled in yield of milk for third lactation animals with the genotype G/G at 10%, as well as the number of milk fat and protein for the third lactation at 9 and 11%, respectively. A significant association of genotype G/A with the underweight calves at birth ($p < 0,05$) was found. Animals with genotype G/G at the first insemination have age in 2 months lower than cows with genotype G/A ($p < 0,05$). Duration of pregnancy in animals with genotype G/G was greater than that of heterozygous with G/A in 6.6 days ($p < 0,05$).

Key words: TNF-a, polymorphism, cattle, milk yield, age at first insemination, pregnancy, birth weight.

Marker and genomic selection in cattle breeding

Use of modern genetic achievements and breeding contribute to accelerate the improvement of existing and creation of new highly productive breeds and types of animals that combine high genetic productivity potential with adaptation to environmental conditions. The rapid development of methods for the analysis of DNA has led to an increase in the number of DNA markers and the

development of the so-called MAS-selection (marker associated selection), accelerating the breeding process, making it more accurate and less expensive¹

Furthermore, the world a genomic selection method has widespread received. The essence of this method is to evaluate the contribution of a large number of uniformly distributed throughout the genome genetic markers in a phenotypic trait on the reference population of animals. In the future, genomic breeding evaluation can be calculated only on the basis of genotyping selection of candidates. However, as a

* To whom all correspondence should be addressed.

result of crossing-over, resulting in the reference population genomic breeding evaluation is necessary to redefine again in a few generations of animals or when selecting a new breed². Upon detection of DNA variants that directly affect the phenotypic trait, the need for such a reevaluation falls away, so the search for such mutations remains an important challenge³

Single nucleotide substitutions and the basic economic useful traits

Among all selected traits in farm animals a significant proportion is for productive signs of having large scale of phenotypic variability. In most cases, they are characterized by polygenic mode of inheritance and controlled by set of genes localized in multiple DNA loci. In hereditary determination of these features distinguish the participation of additive polygenes, oligogen (main effect genes), modifier genes, whose contribution to the formation of quantitative characteristics can vary considerably depending on the trait under study. Despite the high paratypic (environmental) component in the phenotypic trait diversity, proportion of genetic influence allows selective improvement of agricultural populations⁴

One of the main economically useful features is signs of milk production - milk yield, fat and protein content in milk. Links of milk production traits with polymorphisms of kappa casein genes⁵, alpha-lactalbumin⁶, beta-lactoglobulin⁷, somatotropin⁸ and others were set up.

However, the high level of productivity cannot be implemented in poor reproductive ability. Growth in milk production of cows is often accompanied by a decrease in the reproductive functions, that is the main reason for culling animals⁹. A number of studies have established the influence of single nucleotide substitutions (SNP) in genes involved in the functioning of the endocrine and immune systems, impaired fertility^{10, 11}. For example, illustrated the influence on these parameters SNP in the growth hormone receptor gene and the progesterone receptor^{12, 13}.

In addition, one of the most important breeding traits is indicators of growth and development, which affect the formation of the future constitution, exterior, productivity and viability of the animals. The high growth rate in

the early postnatal period is preferable from an economic point of view. Previously studied the association of genes Bhlhe40 (basic helix-loop-helix family member e40), DPP6 (dipeptidyl-peptidase 6) and CLEC3B (C-type lectin domain family 3 member B), and? growth hormone gene with a mass of calves at birth and at weaning average daily gain of native Chinese and Brazilian cattle, as well as in black and white breed of cattle¹⁴⁻¹⁶.

The study of genetic markers at the DNA level continues not only in genes directly affecting the quantitative traits, but also for the genes that are not directly associated with these features, based on their possible linked inheritance or pleiotropic effects of candidate genes.

Justification of the choice of the studied gene

Tumor necrosis factor alpha (TNF- α) is a multifunctional protein belonging to the class of cytokines. Along with the regulation of the immune response, TNF- α regulates appetite, lipid metabolism, stimulates the production of prostaglandins, stimulate alteration and leukocyte adhesion to vascular endothelial cells¹⁷. Also TNF- α takes part in the processes of morphogenesis and development of the fetus, as well as plays an important role in the formation of mammal gland^{18, 19}.

In cattle gene TNF- α is located in the 23 chromosome, in 23q22 locus in the region of the gene cluster of major histocompatibility complex BoLA²⁰. It is shown that a single nucleotide substitution A on G at position -824 of the promoter of the gene TNF- α leads to decrease mRNA expression in cows leukocytes²¹. Thus, the gene TNF- α is a promising candidate gene of principal economically useful traits.

A number of studies demonstrated the association of this gene polymorphism with milk production²², with a level of milk yield, depending on the sex of calf²³, with a weight of calves at birth²⁴, with the timing of the first ovulation after calving²⁵.

The aim of the work is to study the genetic structure of herds of red steppe breed by polymorphism -824 A / G gene in the promoter of the TNF- α gene, and to study possible associations of polymorphism with the main features of milk production, reproduction, growth and development.

METHOD

Blood of 148 cows of red steppe breed MR PP “Stepnoy” Altai Krai was used for analysis. Growth and development, reproduction and milk production indexes are obtained on the basis of zootechnical accounting. Laboratory studies were carried out in the Laboratory of Molecular Human Genetics, Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences. Isolation of genomic DNA was performed using standard proteolytic processing method, followed by extraction with phenol-chloroform. A single nucleotide polymorphism (SNP) gene TNF- α -824 A / G was studied by PCR-RFLP method using the forward primer 5' CCGAGAAATGGGACAACCT-3' and reverse primer 5' -GCCATGTATCCCCAAGAAT-3'.

The amplification of the fragment of 145 base pairs (bp) was carried out in 25 μ l reaction mixture containing 75 mM TrisHCl (pH 9.0), 20 mM $(\text{NH}_4)_2\text{SO}_4$, 0.01% Tween 20, 3 mM MgCl_2 , 200 μ M each dNTP, 1 μ M each primer, 2.5 units activity of Taq-polymerase (SibEnzyme, Novosibirsk, Russia), 6% glycerol and 0.5-1 μ g DNA for 35 cycles (95° C - 30 seconds, 62° C - 30 seconds, 72° C - 30 seconds) with pre-denaturation at 95° C for 3 minutes.

The amplification product was subjected to restriction by enzyme EcoICRI (SibEnzyme, Russia) under the conditions recommended by the manufacturer. Amplicon 145 bp remained uncut

(allele G), or cut into fragments of 81 and 64 bp (allele A).

Electrophoretic analysis of the fragments was performed with 4% *polyacrylamide gel* supplemented with ethidium bromide. Statistical analysis was made by the program Statistica v. 8.0. Assessment of the significance of differences of indicators from animals with different genotypes was performed using analysis of variance (ANOVA). Pairwise comparisons between groups were performed by post hoc analysis using Fisher's exact test and Student's t test.

RESULTS

The study of genotypic structure on the gene TNF- α has shown that the proportion of homozygous G / G and A / A is respectively 56 and 6%, the proportion of heterozygous G / A is 38%. The frequency of allele G is 75%, and allele A - 25%. The overall degree of homozygotes for this gene in a herd is 62%. The frequencies of genotypes in the population were not significantly different from the expected distribution of Hardy-Weinberg equilibrium.

Depending upon the genotype of the gene TNF- α groups of cows were formed and milk production rates over the first, third, average and high lactation were evaluated. Significant differences between cows of different genotypes were detected by the third lactation and are

Table 1. The milk yield of cows of different genotypes by polymorphism -824 A / G gene for TNF- α by the third lactation

Genotype	Milk yield, kg	Milk fat, %	Milk protein, %	Milk fat yield, kg	Milk protein yield, kg
G/G	3766 \pm 87	4,39 \pm 0,03	2,98 \pm 0,01	167,5 \pm 4,1	112,2 \pm 2,9
G/A (4170 \pm 132**	4,41 \pm 0,01	3,00 \pm 0,01	183,7 \pm 6,0*	125,6 \pm 5,0*
A/A (3886 \pm 233	4,39 \pm 0,01	2,97 \pm 0,01	170,6 \pm 10	115,7 \pm 8,0

Note: * - $p < 0,05$, ** - $p < 0,01$ compared to the group G / G

Table 2. The live weight of young calves at different periods of ontogenesis, depending on the genotype of the gene TNF- α

Genotype	at birth, kg	6 months, kg	12 months, kg	18 months, kg
G/G	35,96 \pm 0,57	164,2 \pm 3,7	242,1 \pm 2,5	328,8 \pm 4,6
G/A	34,16 \pm 0,65*	158,0 \pm 1,4	240,4 \pm 3,4	332,2 \pm 3,1
A/A	35,50 \pm 0,50	152,5 \pm 2,5	240,5 \pm 1,5	324,5 \pm 2,5

Note: * - $p < 0,05$, compared to the group G/G

presented in Table 1.

An increase in milk production by 10% in the group of cows genotype G / A compared with milk yield of cows genotype G / G ($p < 0,01$) was found. Except these significant differences, there is a tendency to excess milk production in heterozygous G / A relative to the homozygotes A / A ($p < 0,06$). Although there were no statistically significant differences between cows of different genotypes on the percentage of fat and protein in the milk, the quantity of milk fat and protein for the third lactation in heterozygous G / A was in 9% and 11% higher respectively ($p < 0,05$) in comparison with homozygous G / G.

Results of the study of growth and development of these cows are shown in Table 2. We may note that different animals in different stages of ontogenesis have different parameters depending on the genotype. Thus, the live weight of calves at birth with genotype G / A was significantly lower compared with the homozygous G / G ($p < 0,05$). The trend of superiority of these homozygotes is also observed in 6- and 12-month old calves. In the later period the environmental factor eliminates the differences between genotypes in live weight.

The associations of gene polymorphism of tumor necrosis factor with indicators of reproduction (age at first insemination, service period, duration of pregnancy, interpregnancy and dry period) were analyzed. Significant association is shown in Table 3.

Among the parameters of reproductive ability of cows age at first insemination is allocated, which characterizes sexual and physiological maturity of the body. Comparing cows with different genotypes by this indicator it was revealed

that animals with genotype G / G have a lower value compared with genotype G / A for 2 months ($p < 0,05$) and a trend for earlier insemination than genotype A / A. By analysis of variance a significant effect of genotype on gene tumor necrosis factor alpha on the age at first insemination was revealed ($F_{(2; 144)} = 3,825, p = 0,024$).

For the duration of pregnancy also significant differences between animals with genotype G / G and G / A on the third lactation were revealed, the first exceeds the second by 6.6 days ($p < 0,05$).

DISCUSSION

The genetic structure of the population

Analysis of the genotype frequencies of polymorphism -824 A / G gene TNF- α in a herd of red steppe breed revealed that 38% were heterozygous cows G / A, 56% - homozygous G / G and 6% - homozygous A / A. The frequency of allele G is 75%, and allele A - 25%. With the high incidence of the G allele in the sample, it is possible to assume its selective advantage with respect to allele A. For comparison, in the cows of black-pied breed and gray Ukrainian G allele frequency was 58 and 48%, respectively²³. Increased frequency of G allele was also established at the Yakut cattle (77%) and bison (100%), which may be the result of natural selection of animals living in extreme environmental conditions²³. It is also possible that the increased frequency of allele G in the red steppe breed is associated with the use of a limited number of seed breeding bulls - the carriers of this allele.

The Association gene TNF- α with milk production

Tumor necrosis factor alpha is a multifunctional protein that has immunomodulatory and anti-inflammatory effect, activates hemostasis. As it is known, production of the cows is depended on many factors, including resistance to diseases. After calving activity of leukocytes in cows reduced, there is a general immune suppression, is the cause of mastitis and endometritis²⁶. TNF- α stimulates alteration and leukocyte adhesion to vascular endothelial cells, and thus can affect the development of these diseases. Significant differences between cows of different genotypes were detected by the third lactation milk productivity. Thus, animals with genotype G / A had a milk yield of 10% higher than

Table 3. Age of the first insemination and duration of pregnancy of cows of different genotypes with polymorphism -824 A / G gene TNF- α

Genotype	Age of the first insemination, months	Duration of pregnancy, days
G/G	24,6 \pm 0,5*	283,1 \pm 1,8*
G/A	26,6 \pm 0,6	276,5 \pm 2,4
A/A	26,6 \pm 1,4	286 \pm 6,9

Note: * - $p < 0,05$, compared to the group G / A

animals with genotype G / G ($p < 0,01$), as well as a tendency to exceed the milk yield in compared with animals with genotype A / A ($p < 0,06$). It is known that one of the conditions for sustainable reproduction of the population is to maintain an optimal level of genetic diversity as the basis of natural selection and characteristic genetic distribution of genetic grades for individual loci corresponding homo- and heterozygotes²⁷. Thus a heterozygote can be advantageous to the number of attributes of adaptation, including milk production. Although significant differences in the first, and the highest and average for all lactating cows milk yield between the different genotypes was not established, the fact of the superiority of heterozygotes relative to homozygous in milk yield for the third lactation indicates their possible adaptive advantage. The evaluation results of milk fat and protein of cows depending on genotypes TNF- α improve the conclusions of the analysis of milk production. Although there were no statistically significant differences between cows of different genotypes on the percentage of fat and protein in the milk, the quantity of milk fat and protein for the third lactation in heterozygous G / A was in 9% and 11% higher respectively ($p < 0,05$) in comparison with homozygous G / G.

The Association gene TNF-a with growth and development

Take into account the involvement of tumor necrosis factor in the regulation of appetite, lipid metabolism, and participation in the processes of morphogenesis and development of the fetus the possibility of the link of polymorphism in the gene for TNF- α with growth and development of calves was analyzed. Calves with genotype G / G were born on average 1.8 kg heavier ($p < 0,05$), than calves with genotype G / A. The trend of superiority of live weight was maintained at 6 and 12 months of age. Because single nucleotide substitution in the gene for TNF- α gives a lower level of mRNA expression²¹, and as a result, the probable reduction in the amount of protein, leading to metabolic shifts.

The Association gene TNF-a with signs of reproduction

The effect of polymorphism of tumor necrosis factor alpha on reproductive performance in cows was shown in study Shirasuna *et al.*, which was set the association genotype A / A with a long

service period and the delayed first postpartum ovulation. In our study, link of genotype G / G with an earlier age at first insemination was defined. Therefore, we can assume that animals with genotype G / G reaches the desired for insemination live weight in a shorter period. Previously established the superiority of animals with genotype G / G on live weight at birth, 6 and 12 months is a proof of that. Besides association of this genotype with longer pregnancy compared to heterozygous G / A is shown. Most likely, this is due to the fact that TNF- α stimulates the production of prostaglandins - lipid mediators that act on the endothelium and uterus due at birth at activity.

CONCLUSION

Thus, the versatility of the tumor necrosis factor makes it a promising genetic marker for economically useful traits. The obtained results of the gene polymorphism study indicate of its association with indicators of milk production, weight of calves at birth, age of first insemination and the duration of pregnancy in cattle. The obtained results are preliminary and require confirmation on more numerous samples of animals of different breeds with additional biochemical, morphological and physiological and other methods. If the results of the study are confirmed, it will allow for the inclusion of cows with effective genotypes gene TNF- α in traditional breeding methods of selection to achieve in a shorter time the increase breeding progress on the basis of productivity in cattle herds of milk trend and to use these data in genomic breeding evaluation of animals.

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