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FEATURES OF THE INTERACTION OF NONALLELIC CANDIDATE QTL GENES IN MICROPOPULATIONS OF PIGS OF LARGE WHITE AND MYRGOROD BREEDS

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It has been carried out the pig genotyping for loci OPNin6, ACTN1, FSHβ, PLIN1 and PLIN2. It was showed showed a high level of polymorphism of all used marker systems. Evaluation of associations fertility genes (OPNin6, ACTN1, FSHβ) according to perilipin loci (PLIN1 and PLIN2) showed the existence of significant associations in two variants of combinations of pairs of loci in a sample of Large White breed of pigs (PLIN1-ACTN1 ($p < 0.01$) and PLIN2-ACTN1 ($p <0.01$)) in contrast to the population of Myrgorod breed of pigs, where the association between the genes of studied loci were absent.

Candidate genes, quantitative trait loci, pleiotropy, population, perilipin, actinins, osteopontin, beta-subunit of follicle-stimulating hormone, myrgorod pig breed, large white pig breed

According to modern notions of population genetics, the single micro evaluation event is not the change of allele frequencies of a separate gene but the transition of a balanced system of alleles of different genes from one state into another one that results in the frequencies of integrated phenotypes accordance to living conditions of the population. The selection of agricultural animals for the complex traits that have a selective significance forms the set of integrated phenotypes which are typical for this artificial population. It stipulates in uncasual combination of alleles of genes which are connected with selective traits that is to beginning associations between them in the population. Thus, the interaction of uncoherent, functionally not connected genes is stipulated by the selection for adapted and economical useful traits and does the important contribution into the formation of total structure of the population.

Reasons of the formation of such allele associations in populations can be the off-balance for a cohesion, uncasual segregation of not homological chromosomes, differential vitality of gametes or zygotes and also the interaction of higher given reasons in the process of selecting integrated adapted genotypes Besides, in herds of domestic agricultural animals which conditionally can be considered as relatively secluded micro populations of the limited effective number, the cozegregation of not allele genes is connected with using separated distinguished boars and directed selection of paternal pairs [1] for receiving posterity with desirable level of the display of a spectrum of economical traits.

Allele associations of independent genes in populations can reflect their connection with genetic loci that control complex of traits for which the selection takes place in population and also the intensity and the direction of such selection [2].

Allele associations of uncoherent functionally independent genetic systems more than once were displayed in micro populations of domestic pigs. Described allele associations of Hal gene (stress-syndrome which is stipulated by the sensitivity to halothane) with Ada genes (adenosindeaminase) [3] and alleles of the system of groups of blood *EAG* [4], alleles *EAG* with *Gpi* gene (glucosophosphatisomerase) and *EAH* (the system of groups of blood H) [5] *EAH* gene with alleles of system of groups of blood *EAC* and *EAJ* [6].

Domestic researchers found out reliable associations of separated genotypes for gene of rianodin receptor (*RYR1*) and alleles of genes of estrogene receptor (*ESR1*) and growth hormone (*GH*) for pig breeds that are spread in enterprises of Ukraine [7].

Used loci in our work namely *OPNin6* (gene of osteopontin), *ACTN1* (gene of alfa-actinin 1), *FSHβ* (gene of beta-subunit of follicle-stimulatin hormone) and *PLIN* (gene of perilipin), which were marked on the genetic map of pig. For each of them it was showed the connection with separated adapted and economical useful traits [8]. So, *FSHβ* localized in the 2nd chromosome in boar organism regulates some stages of spermatogenesis and influences on sex behavior; for sows it has been determined the association of some allele variants with increasing multifertility [9]. Locus *ACTN1* is situated on the 7th chromosome and together with loci *ACTN2*, *ACTN3*, *ACTN4* form a family of proteins-actinins that influence on the development of muscle system of pig; Chinese scientists on local breeds of pigs showed the influence of polymorphism 18G>A *ACTN1* on reproductive qualities of boars , specifically indexes of sperm quality [7,10]. Gene of osteopontin is on the 8th chromosome, the polymorphism of which is connected with the deletion in the 6th intron (*OPNin6*) and associated with functional qualities of boar sperm and per cent of embryo implantation in sows of Asian breeds [7]. PLIN-gene, which is situated on the 9th chromosome, has two mutations associated with economical useful traits: it was determined the probable connection of AG genotype for polymorphic site 4119A>G (*PLIN1*) with reduced fat thickness in pigs of Landrace and the Large White breed, AA genotype – with increased average daily gains of an animal; individuals of GG genotype are characterized by undesirable fatty of carcasses at fattening. For the inserciation 7966 T>C (*PLIN2*) it was determined the existence of analogous associations between genotype and a certain fattening trait of pigs, specifically desirable genotypes are determined CT for reducing fat thickness and TT for increasing average daily gain of young pigs at fattening [11]. On the base of higher given data the aim of our work was the search of between loci associations of uncoherent genes of loci of some QTL pigs of the Large White and Myrgorod breeds for the determination of a level of their genetic consolidation for the elaboration of further selective strategy by the help of molecular -genetic markers.

Materials and methods of researches

In the work it was used the data for 49 pedigree animals of Myrgorod breed (SE EE PF named after Dekabrysty) and for 67 pigs of the Large White breed (AF SVK “Orzhytska”, SEEE “Stepne”,PAF “Ukraine”,Ltd “Maiak”).It has been taken the samples of bristles with root bulbs from experimental animals from which then it was extracted genome DNA by the help of reagent “Chelex 100” [12]. Genotyping for loci *OPNin6, ACTN1*, *FSHβ, PLIN1* and *PLIN2* was carried out according to recommendations [8] with using chemical agent from the firm “Fermentas” (Lithuania).

The complex of consider selective significant quantitative traits includes the index of average daily gain, back fat thickness on the level of 6th - 7th pectoral spines and results of control fattening. The statistical processing results of the analysis was carried out by standard methods [13]. As a quantity, that shows the degree of association of discrete quality traits in the population it was used the coefficient of association (tetrahoric index of the connection), the reliability of which was estimated by the x^2 contingency test [14]. All amount of work was performed on the base of the Laboratory of genetics in Institute of Pig Breeding and AIP NAAS of Ukraine.

Results of researches

DNA-typing animals for loci *OPNin6, ACTN1, FSHβ, PLN1* and *PLIN2* showed (table 1), that all researched marker systems are high polymorphic (the level of genetic polymorphism essentially varied depend on the breed of animals and certain locus) that creates possibilities for carrying out the search of between loci associations. For the selection of animals of the Large White breed it was determined the statistic reliable deflection from balanced distribution of genotypes according to the formula Hardy–Weinberg: for locus of osteopontin it was fixed essential surplus of homozigote genotypes BB (p<0.001) that was reflected on the significance of a coefficient of fixation.

Table 1.

Genetic-population testimonial of pigs for loci *OPNin6*, *ACTN1*, *FSHβ*, *PLIN1* and *PLIN2*

Breed	Frequencies					Heterozigote		Index of fixation (F)
	Genotypes (observed/expected)			Alleles		Observed (Ho)	Expected (He)	
	AA	AB	BB	A	B			
				OPNin6				
The Large White	0,065 (0,004)	0,000 (0,122)	0,935 (0,874)***	0,065	0,935	0,000	0,122	1,000
Myrgorod breed	0,000 (0,019)	0,273 (0,236)	0,727 (0,746)	0,136	0,864	0,273	0,236	-0,158
				ACTN1				
The Large White	0,391 (0,322)	0,369 (0,488)	0,240 (0,180)	0,576	0,424	0,370	0,488	0,243
Myrgorod breed	0,455 (0,465)	0,455 (0,434)	0,090 (0,101)	0,682	0,318	0,455	0,434	-0,048
				FSHβ				
The Large White	0,565 (0,612)	0,435 (0,340)	0,000 (0,047)	0,783	0,217	0,435	0,340	-0,278
Myrgorod breed	0,636 (0,669)	0,364 (0,298)	0,000 (0,033)	0,818	0,182	0,364	0,298	-0,222
				PLIN1				
	AA	AG	GG	A	G			
The Large White	0,304 (0,171)	0,218 (0,485)	0,478 (0,3450***	0,413	0,587	0,217	0,485	0,552
Myrgorod breed	0,388 (0,338)	0,388 (0,487)	0,224 (0,175)	0,582	0,418	0,388	0,487	0,203
				PLIN2				
	CC	CT	TT	C	T			
The Large White	0,478 (0,384)	0,283 (0,471)	0,239 (0,145)**	0,620	0,380	0,283	0,471	0,401
Myrgorod breed	0,265 (0,400)	0,735(0,465) *	0,000 (0,135)***	0,633	0,367	0,735	0,465	-0,581

*** - p≤0,001, ** - p≤0,01, *- p≤0,05, criterion χ^2

Analogous tendency in the Large White breed is observed also for the distribution of genotypes for polymorphic sites of perilipin gene (*PLIN1*, *PLIN2*) and a level of inbred animals from total number that were examined and consist of 0.552 and 0.441, accordingly. The advantage of a level of real heterozigotion over expect one for the selection of animals of Myrgorod breed to attract our attention: in results of this it was observed the negative significance of an index of fixation for loci *OPNin6*, *ACTN1*, *FSHβ* and *PLIN2* and for last of counted genetic systems its level was 0.581. This phenomenon can be explained only by selective approach used in the herd of pigs of Myrgorod breed in the pedigree factory named after Dekabrysty. This pedigree enterprise is practically single where this breed of pigs is bred and is on the confines of a disappearance.

It is known that Myrgorod breed for the majority of fattening qualities (particularly for indexes of quickly maturing, the intensity of growth and growth and feed conversion) yields to specialized meat genotypes and even purebred pigs of the Large White breed of English and Danish selection. So, to decrease the level of inbreeding in the herd and also to increase the competitive ability, decreasing the level of loss of housing purebred Myrgorod pigs it was accepted the decision about the creation new genealogical lines at using pigs of ultra meat breed Pietrain and using boars of this breed for receiving young pigs for fattening. Thus, received genetic population parameters in our research confirm the presence among researched animals of fattening live-stock of representatives from new create line Markis (with improved fattening qualities), and also hybrids of different part of blood.

The main idea of carrying out the experiment on search of between loci associations between candidate genes of fattening and reproductive qualities consisted in statistical prognostication of the probability of existence of negative pleiotropy effects that are not physically coherent functionally alternative genes. Because, the exclusion of possible risks of negative influence of a selection of animals for genotypes of perilipin locus on the level of their fertility and the other way round to create prerequisites in reference the possibility of inculcating worked out marker system into the practice of domestic marker selection. Besides, finding out stable associations of genes can be the additional criterion of the breed specificity and a consolidation of the massif of animals.

The analysis of associations of fertility genes (*OPNin6*, *ACTN1*, *FSHβ*) with loci of perilipin (*PLIN1* and *PLIN2*) found out certain statistical reliable connections (table 2). According to obtained results it was showed that reliable associations are observed only in two variants of combinations of loci pairs and only in the choice of pigs of the Large White breed (*PLIN1-ACTN1* (p<0.01) and *PLIN2-ACTN1* (p<0.01)) in contrast to the population of pigs of Myrgorod breed where it was not a failure to find out associations of researches loci by chosen statistical methods.

Table 2.

Between loci associations in populations of the Large White and Myrgorod breeds of pigs

Loci pairs	Coefficient of the associations	
	The Large White breed, n=67	Myrgorod breed, n=49
PLIN1-ACTN1	0,246**	-
PLIN1-OPNin6	-	-
PLIN1- FSHβ	-	-
PLIN2-ACTN1	0,249**	-
PLIN2-OPNin6	-	-
PLIN2- FSHβ	-	-

*** - p≤0,001, ** - p≤0,01, *- p≤0,05, criterion χ^2

This fact, obviously is stipulated by higher level of the genetic homogenization , and so the genetic consolidation of pigs of the Large White breed of research micro population. It has been determined relatively low level of polymorphism which can be stipulated by processes of a single -mindedness selection of better animals for the complex of selective economical useful traits in the enterprise for further their control fattening with using limited number of boars mainly homozigote ones for research genes.

Conclusions and perspectives of further researches.

Obtained data show that in the population of pigs of the Large White breed are reliable associations between markers of independent Mendel genes (*PLIN1-ACTN1* and *PLIN2-ACTN1*), alleles of which correlate with selective quantitative traits of the productivity of pigs. But, not any between genes association for the selection of animals of the Myrgorod breed it was not found out. It testifies about the origin of processes of genetic drift after out breeding in this micro population, that it was pointed over and also by an insufficiency of the number of a choice which was available for carrying out molecular -genetic, statistical and population analysis.

In regard to determined gene associations for pigs of the Large White breed it can foresee the existence of the functional connection of compound physiological nature. According to obtained data (table 1), the exceeded number of animals of a desirable *ACNT1* genotype had and high concentration *PLIN1*-G, *PLIN2*-C alleles, which influence on the quickly fatty of the carcasses. It is the confirmation of well-known fact of better quality of the sperm productivity in pigs of fat and universal direction of the productivity in comparison to animals of ultra meat breeds for which the selection on increasing meat and fattening qualities mainly causes to decreasing some parameters of the fertility namely the quality of sperm and fertilization ability. The determination of such undesirable pleiotropy effects of not alleles loci *PLIN1-ACTN1* can decrease the effect of a selection for simultaneous choice of animals with higher fattening and reproductive qualities. So, the task for a breeder is the necessity for the violation of undesirable between genes associations. In this aspect the better effect from using marker selection for

PLIN genotypes can wait in pigs' herd of Myrgorod breed but this admission needs the additional genetic-statistical researches on enlarged choice of animals.

It is necessary to point that the presence of the association *PLIN1-ACTN1* can be connected with the influence of both genes on the intensity of a growth of pigs – first of them controls fat exchange, and the second is belonged to the cascade of genes-actinins that cause the growth of muscle tissue. So, the character of found out between genes interactions can be explained by the presence of polymerization of not allele genes *PLIN1-ACTN1* and pleiotropy effects for *ACTN1* locus, because it influences simultaneously on the indexes of fattening and reproductive qualities.

It is necessary to note that associates of researches of genetic markers of polymorphic systems, obviously must be presented in other populations of pigs which belong to intensive selection. They are an important testimonial of populations that reflect not accident of processes of the combination of allele variants of independent genes at the formation of morphotype which adequately responds to conditions of the selection. The existence of associations of markers of Mendel genes in the population can also point on the coherency of marker genes with genetic loci that are determining the formation of the complex of quantitative traits for which is carried out the selection.

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