

Original Research Paper

The *COXII* Haplotypes and their Association with Productive Traits in Large White Breed Pigs

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Abstract: The mitochondrial genome encodes key electron transfer chain (ETC) proteins which produce the vast majority of cellular ATP. Sequence analysis of maternally inherited mitochondrial DNA is an effective way to assess the individual characteristics of commercial breeding lines of pigs. The aims of our study were to analyze the nucleotide sequence of the mitochondrial gene *COXII* in Large White pigs, to search for polymorphisms and to evaluate their associations with pigs productive abilities and the backfat thickness. The studied pigs of Large White were divided in two groups. The first group (LW-1) was consisted of pigs bred in Russian Federation since 1998. The second group (LW-2) included pigs of Large White imported in 2014 to Russia from England. To evaluate the associations of the *COXII* gene haplotypes with productive qualities, 96 sow LW-1 were selected. The variability analysis of the *COXII* revealed the presence of three haplotypes: Hap_1 (TTTCTGGAT, in positions m.8292, m.8334, m.8419, m.8466, m.8526, m.8634, m.8664, m.8682, m.8682, m.8694 respectively), Hap_2 (CCCCAAGC) and Hap_3 (TTTTTGGAT). We have obtained the results on the association of the *COXII* polymorphic variants in the loci (m.C8292, m.C8334, m.C8419, m.C8526, m.A8634, m.A8664, m.G8682, m.C8694) that are typical for Hap_2, with higher value of backfat thickness in pigs.

Keywords: MtDNA, *COXII*, Nucleotide Sequence, Gene Position, Haplotypes, Pig, Productive Qualities, Backfat Thickness

Introduction

Mitochondria play an important role in metabolic processes as well as in apoptosis and cell aging. Polymorphism identified in mitochondrial DNA is one of the most common genetic markers used in population studies of many animal species. It's caused by the high rate of mtDNA mutations and the fact of the strict maternal fashion inheritance of mitochondria.

Both male and female individuals get mitochondria from the cytoplasm of the maternal egg. MtDNA is not able to recombination, the entire molecule is changed only by mutation for thousands of years (Fernandez *et al.*, 2008; Nisztuk-Pacek *et al.*, 2018). Pigs mtDNA is an annular molecule consisting of average of 16.5 kbp. In pigs it includes 37 genes: 13 for proteins of the Electron Transfer Chain (ETC), 22 for tRNA and two rRNAs (16S rRNA and 12S rRNA), as well as the most variable region, the D-loop (Yen *et al.*, 2007).

For billions of years, the mitochondrial genome has evolved as organisms have adapted to the environments (Srirattana *et al.*, 2017). As a result, mutations have become fixed. Various mitochondrial lineages have arisen from one or several common sources – mitochondrial genomes, which are characterized by clustering into groups known as mtDNA haplotypes (Wallace *et al.*, 2003). Consequently, mtDNA haplotypes cause differences between breeds and are associated with both advantages and disadvantages to the organism (Wallace *et al.*, 2003).

In cattle associations with calving (Sutarno *et al.*, 2002), meat quality (Mannen *et al.*, 2003), milk (Bell *et al.*, 1985), embryo production efficiency (Tamassia *et al.*, 2004; Bruggerhoff *et al.*, 2002) and body weight (Derr *et al.*, 2012) were identified. In pigs associations with body weight (Yen *et al.*, 2007) and meat quality (Fernandez *et al.*, 2008) was discovered. Furthermore, the associations with the reproductive capacity and litter size in

pigs have been reported (Tsai *et al.*, 2016; Justin *et al.*, 2018) and productive and reproductive traits in Afec-Assaf sheep (Reicher *et al.*, 2012).

The vast majority of the mitochondrial genome mutations belongs to the class 1 (silent mutations (third codon)), while the mutations of class 2 (first and second positions of codons) lead to an amino acid substitution. In the genes that encode the mitochondrial proteins the observed frequency of substitutions is much higher for the sites of class 1 than for the sites of class 2. This is true for the gene of subunit II of cytochrome oxidase C (*COXII* gene). The sites of class 1 accumulate substitutions faster than sites of class 2.

The mtDNA gene *COXII* is one of the electron transfer chain complex IV elements. Due to this fact it plays a key role in energy production. Therefore the mutations in the *COXII* gene can be associated with economically important characteristics of pigs. Taking into account the role of the gene and the relatively high degree of intra-species variability, the identification of polymorphisms in pigs mtDNA and the nucleotide sequence investigation of the *COXII* gene are very prospective (Liu and Lizhi, 2017; Nisztuk-Pacek *et al.*, 2018).

The aim of this study was the investigation of the mtDNA *COXII* gene nucleotide sequence in Large White pigs, the identification of polymorphisms and the evaluation of their associations with the productive qualities of pigs.

Materials and Methods

To determine the mitochondrial *COXII* gene nucleotide sequence samples were collected from two groups of Large White pigs. The first group consisted of Large White pigs (LW-1) bred in Russian Federation since 1998. The second group (LW-2) included pigs of a Large White imported from England to Russia in 2014. The mtDNA was isolated from 48 tissue samples of LW-1 (n = 24) and LW-2 (n = 24) groups using the “K-Sorb-100” kit (Syntol, Russia) according to the manufacturer’s instructions.

The sequence NC_000845.1 (*Sus scrofa*) published in the NCBI has chosen as reference. The *COXII* was amplified using forward (5'-CACGGATGTCCTCCTCCCTA-3') and reverse (5'-TTCTGGGCTTGCTGGGTATG-3') primers. The 25 µl reaction mixture consisted of 3 µl of DNA sample (75ng/µl), 5 µl of 10 × PCR-standard reaction buffer, 1 µl dNTP (1 mmol/l), 0.5 µl of each primer (20 µmol/l) and 0.5 µl Taq DNA polymerase. The amplification conditions were as follows 94°C for 3 min followed by 33 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 1.5 min. The final step was 72°C for 5 min.

Specific PCR fragments were isolated from the gel using the Cleanup Mini kit for the DNA purification

from the gel (Evrogen, Russia). Amplicons were sequenced using a Terminator Cycle Sequencing kit (Applied Biosystem, Foster City, CA) in the Gene Amp PCR system 9700 (Applied Biosystem) BioEdit. For editing and sequences alignment BioEdit v7.2.6 and MEGA 7 software were used.

To evaluate the associations of the *COXII* gene haplotypes with productive qualities, 96 sows LW-1 were selected. Productive qualities were assessed by Backfat thickness (BF) and Average Daily Gain (ADG), measured when the animals weight reached 100 kg. Analysis of the *COXII* polymorphism effect on the sows productive characteristics was performed using LMM (Linear Mixed Models) (Getmantseva *et al.*, 2017; Harrison *et al.*, 2018). The parameters of the mixed linear model were calculated using the lme4 package of the R language in the R-studio environment.

Results

At the first stage, 48 nucleotide sequences of the *COXII* gene were studied, 24 of the LW-1 group and 24 of the LW-2 group. As a result the nucleotide sequence of the *COXII* gene was determined. When analyzing the obtained data, it can be noted that the position of the *COXII* gene is within the interval of 8203.. 8890 bp, the size of the gene is 687 bp, which corresponds to the reference population. The studied sample includes three haplotypes, which formed by 9 substitutions in the positions m.8292, m.8334, m.8419, m.8466, m.8526, m.8634, m.8664, m.8682, m.8682, m.8694 (Table 1).

Two haplotypes (Hap_1 and Hap_2) were identified in the LW-1 pigs. All the animals from the LW-2 group had only Hap_3. It is interesting to note that Hap_1 which have been defined for LW-1 differs from Hap_3 by only one substitution in the m.8466 locus. At the same time, Hap_2, belonging to LW-1, also differs from Hap_3 by substitution in the m.8466 locus and by substitutions in 8 other loci. Intra-group differences in LW-1, presented by Hap_1 and Hap_2, are due to substitutions in 8 loci: m.T8292C, m.T8334C, m.T8419C, m.T8526C, m.G8634A, m.G8664A, m.A8682G, m.T8694C, respectively.

To evaluate the associations of the *COXII* gene haplotypes with productive traits, the LW-1 sample was increased by 96 pigs. Figures 1 and 2 shows the distribution of the BF and ADG values of different haplotypes in LW-1.

Figure 1 shows that BF in pigs with Hap_1 is distributed symmetrically and has greater variability than Hap_2. It should be noted that there is a very low variety of high BF values in Hap_2. Comparison of Hap_1 and Hap_2 graphs allows us to suppose significant differences between them in the value of BF.

Table 1: Haplotypes of the COXII in large white pigs

Haplotype	m.8292	m.8334	m.8419	m.8466	m.8526	m.8634	m.8664	m.8682	m.8694
Hap_1	T	T	T	C	T	G	G	A	T
Hap_2	C	C	C	C	C	A	A	G	C
Hap_3	T	T	T	T	T	G	G	A	T

Table 2: Differences in BF and ADG in pigs of LW-1 with Hap_1 and Hap_2 of COXII

Trait	Hap of the <i>COXII</i>		df	χ^2	p
	Hap_1	Hap_2			
BF	20.86±1.02	24.01±1.58	1	4.896	0.026*
ADG	783.02±22.43	764.02±27.68	1	0.586	0.443

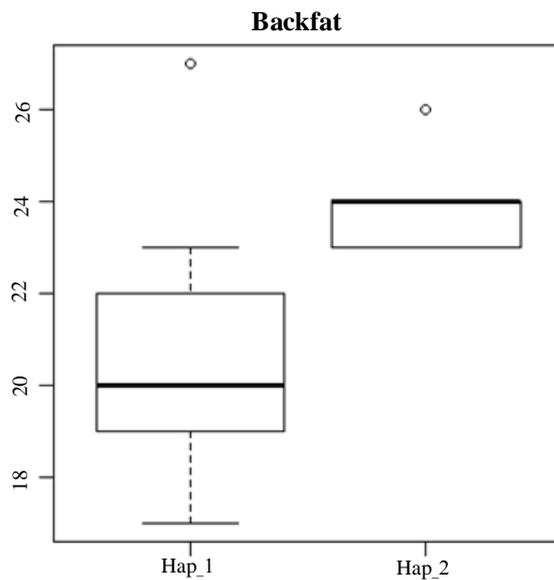


Fig. 1: Backfat thickness in pigs of the COXII Hap_1 and Hap_2

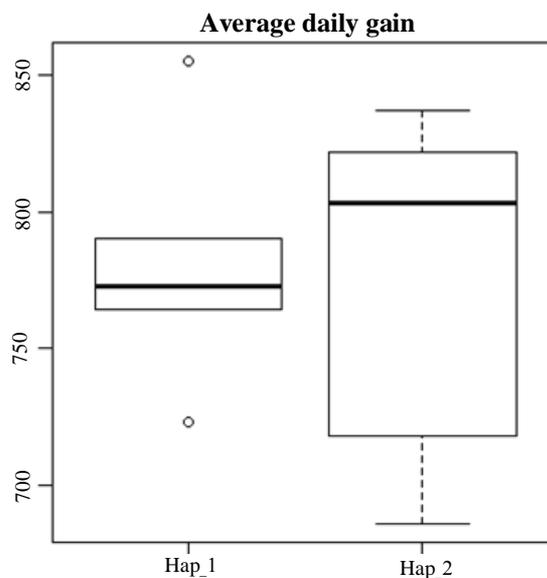


Fig. 2: Average daily gain in pigs of the COXII Hap_1 and Hap_2

According to the graphs shown in Fig. 2, the Hap_2 group shows a high variability in the ADG compared to Hap_1.

The reliability of differences between the Y traits (BF and ADG) which are associated with the *COXII* haplotypes was assessed using Linear Mixed Model:

$$Y_{ijk} = \mu + Hap_i + Year_j + P_k + e_{ijk}$$

The fixed factors were Hap (Hap_1 and Hap_2 of the *COXII*) and the Year of the animal. A randomized factor was the sire's number (P - parent). As a result the following full_model on the syntax R was used:

$$Y \sim Hap + Year + (1|P)$$

To evaluate the conditionality of BF and ADG by factor Hap we compared full_model to null_model:

$$Y \sim 1 + Year + (1|P)$$

The obtained results have shown the significant effect of *COXII* haplotypes on backfat thickness (Table 2). Hap_1 was associated with a lower BF. The differences in ADG caused by factor Hap of the *COXII* in pigs of LW-1 group didn't reveal.

Discussion

Studies of the polymorphism of nuclear and mtDNA allow us to uncover the unique biological characteristics of pigs and develop selection methods using molecular genetic information. Today, data on mtDNA of the main breeds are available, but the search for associations of mtDNA polymorphism with productive qualities of pigs is of increasing interest.

At present, the study of the complete mitochondrial genome of pigs is given much attention (Tsai *et al.*, 2016; Nguyen *et al.*, 2016; Justin *et al.*, 2018; Kumar *et al.*, 2019; Yang *et al.*, 2019). The results of Tsai *et al.* (2016), which identified five haplotypes of mtDNA in commercial lines of Australian pigs. Their studies allowed to determine the genetic diversity of domestic pigs and to show how mtDNA haplotypes influenced Litter size, as well as ovulation rate, uterine efficiency and embryo survival. Studies by Justin *et al.* (2018) also demonstrated the effect of mtDNA haplotypes on fat density, muscle depth, fat-to-weight ratio, daily lifetime increase and nipple quality in pigs. However, few studies have been carried out on individual mtDNA genes and their relationship to pig traits. Due to the fact that *COXII* plays a key role in energy production, it is an interesting object for research.

The results indicating the relationship between the polymorphism of the *COXII* gene were obtained in the study of other species. (Nisztuk-Pacek *et al.*, 2018) have obtained results, which proved the associative connections between the *COXII* gene polymorphisms and phenotypic

characteristics of animals. They pointed to the statistically significant relationship of the mtDNA gene *COXII* haplotypes and haplogroups with the productive traits of raccoon dogs, such as weight, body size and colour type. Jeon *et al.* (2005) showed significant differences between genotypes identified in the mitochondrial gene *COXII* and weight in Hanwoo cattle. Results from the Ślaska *et al.* (2016) indicate that the *COXII* mitochondrial gene may be involved in hair development. The results we have obtained reflect the association of the *COXII* gene polymorphisms with the backfat thickness in pigs.

According to our data, these are the first studies aimed at finding significant associations between the polymorphism of the mitochondrial gene *COXII* and the productive qualities of pigs. We determined that Hap_1 in pigs LW-1 is associated with a lower backfat thickness. It is interesting to note that in the LW_2 group, only Hap_3 is identified. Meanwhile the backfat thickness in LW-2 (BF = 14.5 ± 0.65) is significantly less comparing to LW-1 (BF = 22.2 ± 0.75). The reason of the difference between Hap_1 and Hap_3 is the substitution in a position m.C8466T. Also it may indicate that *COXII* polymorphic variants (in the positions m.C8292, m.C8334, m.C8419, m.C8526, m.A8634, m. loci. A8664, m.G8682, m.C8694), which are typical for Hap_2, are associated with higher backfat thickness in pigs.

The data obtained will complement the knowledge of the molecular genetic mechanisms of the formation of productive features of pigs and can be used in the development of technology for creating breeding resources in pig breeding based on molecular genetic information. In general, the results presented by a number of scientists reveal that mtDNA haplotypes and haplogroups, including the D-loop sequence, *COXI*, *COXII* genes, tRNA and others, are associated with the productive traits of animals. Further studies in this field will assess the value of mtDNA variants as animal productive indicators as well as their using with nuclear polymorphisms for improvement of agricultural animals.

Conclusion

The variability analysis of the mtDNA *COXII* gene revealed the presence of three haplotypes in large white breed pigs: Hap_1 (TTTCTGGAT, in positions m.8292, m.8334, m.8419, m.8466, m.8526, m.8634, m.8664, m.8682, m.8682, m.8694, respectively), Hap_2 (C CCCCAAGC) and Hap_3 (TTTTTGGAT). The results on the association of the *COXII* gene polymorphisms in loci m.C8292, m.C8334, m.C8419, m.C8526, m.A8634, m.A8664, m.G8682, m.C8694, which are typical for Hap_2, with higher backfat thickness in pigs have been obtained.

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Author's Contributions

Maria A. Kolosova, Lyubov V. Getmantseva: Designed and performed experiments and wrote the paper.

Necruz F. Bakoev, Anatoly Yu. Kolosov, Nare A. Akopyan and Varvara S. Shevtsova: Developed analytical tools and analyzed data.

Alexandr I. Klimenko, Olga V. Kostyunina: Collected and analyzed data.

Ethics

This article is original and contains unpublished materials. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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