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The potential role of MYOM1 and ATGL genes in pig production improvement

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Short title: MYOM1 and ATGL as a gene markers in pig breeding

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Abstract
In the present study, two missense variants within \textit{ATGL} (rs331307082) and \textit{MYOM1} (rs326001585) genes were tested for their potential usage as genetic markers related to pig production traits. The genotyping was performed on 519 pigs representing 990 synthetic sire line. The association analysis indicated that \textit{ATGL} gene affected the panel of fattening parameters (test daily gain, age at slaughter), meatiness traits (meat percentage in the carcasses; the weight of loin, ham and primary cuts, and loin eye area), and meat quality characteristics (water exudation). In turn, \textit{MYOM1} polymorphism was related to loin weight, the weight of primary cuts and weight of loin backfat. Pigs with AA genotype were characterized by significantly higher loin and primary cut weights compared to opposite homozygotes GG (\textit{p}<0.05). The observed differences were 2.29 kg and 1.2 kg, respectively. Moreover, despite higher meatiness, AA animals together with AG were characterized by lower weight of loin backfat (\textit{p}<0.05) and average backfat thickness (\textit{p}<0.1) compared to GG pigs. The \textit{MYOM1} polymorphism did not affect pork quality traits. The results allowed us to propose the new genetic markers which may be used in pig selection to obtain appropriate meatiness and fatness level in carcasses without decreasing meat quality.

**Key words:** myomesin-1, adipose triglyceride lipase, \textit{PNPLA2}, selection, genetic marker

One of the major challenges of modern pig farming is selection focused on improving specific traits (e.g. meat quality) without deterioration of another group of features. The breeding programs and intensive, one-sided selection aimed to increase meat characteristics that resulted in a significant decrease in pork quality. On the other hand, attempts to enhance pork quality have resulted in losing a satisfactory level of meatiness obtained during long-term selection (Orzechowska and Tyra, 2010). Thus, scientific efforts are made to identify
and apply genetic markers which will allow performing selection toward modification of one trait without changing levels of the others.

Despite trying to apply genomic selection in the commercial pig breeding (Knol and Nielsen, 2016), the single genes are still being tested for their usefulness in breeding work and importance for accelerating the livestock progress. The limitation of genomic selection in pigs is still a relatively high cost of single analysis (Samorè and Fontanesi, 2016). The high-throughput SNP data obtained by RNA sequencing was applied to identify the set of genes with a potential high effect on essential production traits in the pig industry (Piórkowska et al., 2018). Among others, two missense variants within MYOM1 and ATGL genes were detected and proposed as a candidate for carcass parameters, backfat thickness and selected fattening traits (Piórkowska et al., 2018). The MYOM1 gene encoding for myomesin-1, is responsible for sarcomere assembly via stabilization of the three-dimensional conformation of the thick filaments (Lange et al., 2005). Myomesin, together with obscurin and obscurin-like-1 proteins form the M-line and are responsible for the regulation of force distribution exerted by the thick filaments during contraction (Fukuzawa et al., 2008). Moreover, myomesin in a complex with myosin and titin anchors thick filaments in the A-band (Agarkova and Perriard, 2005). In turn, the ATGL gene (also known as PNPLA2) encodes adipose triglyceride lipase, which catalyzes the degradation of triacylglycerols of lipid droplets in the adipocytes and non-adipocyte cells (Zimmermann et al., 2004). This lipase is involved in energy homeostasis by playing a role in the body's response to starvation. Under conditions of reduced caloric intake, ATGL activates triglyceride hydrolysis ensuring delivery of free fatty acids to other tissues, which can be used as an energy source (Smirnova et al., 2006).

Taking into account the important function of both genes and previous findings, we hypothesized that MYOM1 and ATGL genes could be considered as potential genetic markers related to pig production traits. In the present study, the novel, mid-density SNP genotyping
method using the Quant Studio 12K Flex Real-Time PCR System with the Open Array Block was applied to determine genotypes within MYOM1 and ATGL genes. The suitability of MYOM1 and ATGL as markers was tested in pigs of 990 line that were selected for meat content and improved fattening traits.

**Material and methods**

**Animals**

The analysis was carried out on 519 female pigs representing 990 synthetic line. The pigs were characterized by low relationship (4.20 individuals per sire and 1.54 per dam). The analyzed pig line was established in Poland in the 1970s based on six breeds (Polish Large White, Belgian Landrace, Duroc, Hampshire, Landrace of German and British origin) to obtain the top sire line for crossing in pig breeding. The 990 individuals are used in the pig meat industry to produce fatteners characterized by high meatiness parameters together with good meat quality characteristics and fattening performance (Różycki and Dziadek, 2012). In the Polish populations of sire breeds, the 990 line ranks second in terms of usage behind the Pietrain pigs.

All investigated pigs were maintained at two breeding stations under the same environmental and feeding conditions. Pigs were kept in individual pens, fed *ad libitum* from 30 kg to 100 kg (±2.5 kg) and weighed daily throughout the fattening period. When pigs reached 100 kg (±2.5 kg), they were slaughtered, and after 24 h of chilling, right half-carcass was dissected. For each animal the panel of traits has been evaluated: test daily gain (g/day); feed: gain ratio (kg/kg) and age at slaughter (day); carcass yield (%); ham / loin weight (kg); the weight of primary cuts (kg); loin eye area (cm²); meat percentage in carcass (%); ham weight without fat and skin (kg); average backfat thickness (cm); the weight of loin backfat (kg). Moreover,
the meat quality characteristics were measured: meat color (L* – lightness, a* – redness, and b* – yellowness; Minolta CR-310 spectrophotometer), pH estimated in loin and ham muscles (*longissimus dorsi* and *seminembranosus*; respectively) measured at 45 min (pH45) and 24 h (pH24) after slaughter (pH-metr pH-STAR CPU; Matthäus, Pöttmes, Germany), IMF – intramuscular fat content (the Soxhlet method), and WE – water exudation (the Grau–Hamm method). The exact procedure of fattening parameters, slaughter procedure and production traits evaluation was described previously (Żak and Tyra, 2013). All pigs were free of *RYR1*1843T allele.

**Genotyping**

The allelic discrimination method was used to determine *ATGL* c.392G>A and *MYOM1* c.2539G>A polymorphism. The TaqMan MGB Assays were designed for rs331307082 (*ATGL*) and rs326001585 (*MYOM1*) (Table 1) on the Custom TaqMan® OpenArray® Real-Time PCR Plate with Genotyping Assays (ThermoFisher Scientific, product id 4471115). Each DNA sample (2.8 µl) was mixed with the TaqMan™ OpenArray™ Genotyping Master Mix (2.8 µl). The 47 DNA samples and one negative control (both with Master Mix) were placed on each OpenArray (OA) plate with the assistance of the OpenArray™ AccuFill™ System (Applied Biosystems). Next, the OA plates were run in the Quant Studio 12K Flex Real-Time PCR System (Applied Biosystems) with the OpenArray Block according to standard manufacturer protocol. The results were checked and analyzed in the TaqMan Genotyper Software v1.4 (Applied Biosystems) after the OA plate’s quality analysis (QC image).

**Statistical analysis**

The association between evaluated traits and both SNPs in *ATGL* and *MYOM1* genes were estimated using General Linear Model (GLM) procedure with Tukey’s test (SASv.8.02),
and the model was: $Y_{ij} = \mu + d_i + b_j + a(x_{ij}) + e_{ij}$, where $Y_{ij}$ is the observation, $\mu$ is the overall average, $d_i$ is the fixed effect of the $i$ genotype, $b_j$ is the fixed effect of the $j$ test station, $a(x_{ij})$ is the covariate with the weight of the half-carcass for the performance of carcass traits only and $e_{ij}$ is the random error. To estimate significant differences between means, the least square means (LSM) test was used. All results are shown as LSM±SE.

Results

Genotype and allele distribution

The allelic discrimination method allowed identifying all three genotypes for both $ATGL$ c.392G>A and $MYOM1$ c.2539G>A polymorphisms (Figure 1). The call rate for $ATGL$ was 99.4% and for $MYOM1$ 99.0%, respectively.

For $ATGL$ polymorphism, the most abundant were GG pigs (72%), while the opposite homozygotes were only 8%. Similar genotype distribution was obtained for $MYOM1$ c.2539G>A where GG pigs represent 64% of the whole population and AA genotype was carried by 5.4% of the pigs. Minor allele frequency (MAF) was 0.18 for $ATGL$ and 0.21 for $MYOM1$ polymorphisms. According to both polymorphisms the analyzed population was in Hardy-Weinberg equilibrium.

Association with production traits

$ATGL$ c.392G>A polymorphisms

The c.392G>A missense variant within $ATGL$ gene showed significant association with all analyzed slaughter traits corresponding to meatiness: meat percentage in the carcass (%); the weight of loin, ham and primary cuts, and loin eye area (Table 2). Compared to
opposite homozygotes, the AA pigs had 3.7 cm² greater loin eye area (p<0.01); 0.25 kg heavier ham weight and 1.1% higher percentage of meat in carcasses (p<0.05). Both AA and AG genotypes were characterized by the highest values of loin weight (p<0.05) and the weight of primary cuts (p<0.01). Moreover, the AA pigs were characterized by lower average backfat thickness compared to both AG and GG animals. Taking into account fattening parameters, AA animals had lower average daily gain and at the same time higher age of slaughter (p<0.05) (Table 3). Among all analyzed meat quality traits, the analyzed polymorphism within ATGL gene was significantly associated with water exudation (Table 4).

**MYOM1 c.2539G>A polymorphism**

The statistical analysis showed that MYOM1: p.Val847Ile polymorphism affected loin weight, the weight of primary cuts and loin backfat. Furthermore, for average backfat thickness the trend was observed (p<0.1) – GG homozygote pigs were characterized by thicker backfat compared to heterozygotes AG (Table 2). Pigs with AA genotype were characterized by a significantly higher loin of primary cut weights compared to opposite homozygotes GG (p<0.05). The observed differences were 2.29 kg and 1.2 kg, respectively. Moreover, despite higher meatiness, AA animals together with AG were characterized by lower weight of loin backfat (p<0.05) and average backfat thickness (p<0.1) compared to GG pigs. The p.Val847Ile polymorphism within MYOM1 gene did not affect fattening traits as well as meat quality characteristics (Table 4).

**Discussion**

The development of research methods in molecular biology has been closely related to the advances in agriculture, both in livestock breeding and in plant cultivation. The efforts are made to apply the new developing research methods to allow identifying candidate genes and
mutations related to quantitative traits of breeding importance. The standard selection assigned with the use of DNA marker linkage with production traits has a great potential in increasing the sustainability and efficiency of animal production (Beuzen et al., 2000).

Both investigated genes, \textit{ATGL} and \textit{MYOM1}, were previously proposed as candidate genes in pig genome associated with meat quality and carcass characteristics (Piórkowska et al., 2018). Variant calling based on RNA-seq data allowed us to show the significant polymorphisms within transcripts potentially related to pig production traits. Among them, two missense variants were identified - \textit{ATGL}: p.Arg131His and \textit{MYOM1}: p.Val847Ile. Even though presented genes are playing an important role in the processes related to lipid metabolism and energy homeostasis (\textit{ATGL}) as well as skeletal muscle development (\textit{MYOM1}), they have not been widely investigated in terms of pig production traits. The recent report by Piórkowska et al. (2018) indicated that these genes could determine selected phenotypic data in pigs.

The present study showed that polymorphism within \textit{ATGL} gene was significantly associated with key carcass characteristics: loin eye area, ham and loin weights, meat percentage in carcasses, backfat thickness, and selected fattening traits. The pigs with AA genotype were characterized by the highest values in most of the presented parameters and the lower backfat thickness, while they did not differ in meat quality from the other genotypes. This is important finding since the use of c.392G>A missense variant in breeding practice can allow selecting pigs to improve meat content without decreasing its quality. However, there is one limitation of selection toward c.392G>A, because AA animals presented lowered daily gains. According to current trends in pig breeding, the fattening traits are the most decisive economical features in pig production (Mirilović et al., 2016). Although AA pigs had lower daily gains and the highest age of slaughter, detected differences between opposite homozygotes were low (54 g and 5.8 days, respectively). Based on such comprehensive
analysis, we hypothesized that using ATGL SNP can bring benefits in the possibility of improving carcass meatiness in terms of low fat and maintaining meat quality at current levels. The study of Piórkowska et al. (2018) performed on native Puławska and Polish Landrace pigs, confirmed that AA animals in terms of c.392G>A SNP were characterized by lower backfat thickness. In pigs, the transcription analysis of ATGL gene showed its overexpression in backfat tissue compared to other tissues (Dai et al., 2011). Moreover, the association study performed for c.392G>A polymorphism strongly indicated that it affects fat deposition and carcass characteristics in pigs (Dai et al., 2011). Similarly, as in the current study, the authors observed that homozygote pigs (AA) were characterized by higher meatiness parameters (loin eye area, lean meat percentage) and lower fatness (average backfat thickness). Authors proposed c.392G>A polymorphism as a candidate marker for fat deposition traits in pigs. The significant relation of ATGL gene with pig production traits was also pinpointed by Dai et al. (2016), who showed that polymorphisms localized upstream 5’UTR region of ATGL gene could influence meat and fat contents.

In turn, for MYOM1 gene our results as well confirmed a significant effect of c.2539G>A polymorphism on evaluated pig production parameters. Likewise, for ATGL gene, for c.2539G>A SNP one genotype – AA was characterized by significantly higher meatiness traits (loin weight and weight of primary cuts) and lower average backfat thickness (trend observed). The most important finding allowing the potential use of this missense variant in breeding practice is a fact that we observed positive association of c.2539G>A MYOM1 with carcass features and lack of the effect on pork quality and fattening traits. Such an approach allows performing one-side selection to improve specific carcass traits without deterioration of the other pig production characteristics. The above breeding strategy can be applied for pig breeds which represent an insufficient level of meatiness, but desirable meat quality. The significance of MYOM1 polymorphism with backfat thickness, intramuscular fat content and
pork texture parameters were previously indicated (Piórkowska et al., 2018). According to involvement in sarcomeric structure, myomesin 1 is one of the proteins determining the muscle fiber type (Agarkova and Perriard, 2005). It has been established that muscle fiber distribution due to differences in energy metabolism, lipid and glycogen storage strongly affects pork quality (Karlsson et al., 1999) and growth performance (Lee et al., 2016). Moreover, the disruption of each protein (including myomesin 1) involved in M-line structure can result in the degeneration of the sarcomere (Fukuzawa et al., 2008; Prill et al., 2019). In the present research, the significant association of c.2539G>A polymorphism with lean meat characteristics was detected. It can be related to a specific function of myomesin-1 and obtained correlation suggested that MYOM1 should be investigated for use in breeding practice.

In pig breeding, there is still a need to search for single genes with a high effect on phenotypic traits due to the relatively low cost of such marker evaluation for one individual. The present study performed on synthetic pig line 990 that is used as a significant sire component, allows us to propose two polymorphisms within MYOM1 and ATGL genes which may be used in pig selection to obtain appropriate meatiness and fatness level in carcasses without decreasing meat quality. The genetic marker assisted selection can bring measurable financial benefits in a shorter time and in a more precise way than selection based only on phenotypic features. However, further study should be performed to confirm such a correlation in the other pig breeds.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval and consent to participate
The research was performed on biological material derived from pigs maintained and slaughtered in the Test Pig Station (National Research Institute of Animal Production). In the Test Station pigs are slaughtered, dissected and after carcass evaluation, meat is standard intended for consumption. Therefore, our research does not require the approval of the Animal Experimentation Committee.

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Table 1. The detailed SNPs information, genotyping primers and probes.

| Gene  | ATGL (PNPLA2) | MYOM1 |
|-------|---------------|-------|
| SNP ID| rs331307082   | rs326001585 |
| Location| chr. 2:515397 | chr. 6:103536375 |
| Strand | forward      | forward |
| Variants | G/A          | G/A |
| Reference| ENSSSCT000000014036.4:c.392G>A | ENSSSCT00000004091.4:c.2539G>A |
| Primers  | F: TCCGACGGCGAAAATGTCAT | F: GGGCTGAATACAGGTATTACGT |
|         | R: GGCACCCACCTGGATGAG | R: GCTTTGATGTTGGCTTCTCCAT |
| TaqMan MGB probes | CATAACCGCTTCCGCC (VIC) | ACTATCGTGGAAAGTAATTG (VIC) |
|           | CATAACCCACTTCCGCC (FAM) | CTATCGTGAATAATTG (FAM) |
Table 2. The significant association of MYOM1 and ATGL polymorphisms and carcases characteristics.

| Traits                        | MYOM1 c.2539G>A genotypes | ATGL c.392G>A genotypes | GLM significance |
|-------------------------------|-----------------------------|--------------------------|------------------|
|                               | AA  | AG  | GG  | AA  | AG  | GG  | MYOM1 | ATGL |
| Loin weight (kg)              |     |     |     |     |     |     |       |      |
|                               | 10.56±0.95 a                   | 7.96±0.38 b              | 8.27±0.27 b         | 8.01±0.74 AB        | 9.52±0.46 A       | 7.92±0.26 B        |       |      |
| Loin eye area (cm²)           |     |     |     |     |     |     |       |      |
|                               | 52.7±0.1                         | 52.0±0.4                   | 52.1±0.3            | 55.3±0.9 A          | 52.6±0.6          | 51.6±0.3 B         |       |      |
| Weight of primary cuts        |     |     |     |     |     |     |       |      |
|                               | 23.9±0.4 a                         | 23.1±0.2 ab                 | 22.7±0.1 b           | 23.4±0.3 ab          | 23.4±0.2 a         | 22.7±0.1 b         | **    | ***  |
| Meat percentage in carcass (%)|     |     |     |     |     |     |       |      |
|                               | 58.6±0.5                         | 58.1±0.2                   | 57.7±0.1            | 58.7±0.4 a          | 58.0±0.2 ab        | 57.6±0.1 b         |       | **   |
| Ham weight without fat and skin (kg) |     |     |     |     |     |     |       |      |
|                               | 8.77±0.10                        | 8.69±0.04                   | 8.64±0.03            | 8.88±0.08 a          | 8.70±0.03          | 8.63±0.02 b         |       |      |
| Average backfat. thickness (cm) |     |     |     |     |     |     |       |      |
|                               | 1.86±0.06 ab                      | 1.85±0.02 b                 | 1.92±0.01 ab         | 1.78±0.05 b         | 1.90±0.03 ab       | 1.91±0.02 a         |       |      |
| Weight of loin backfat (kg)   |     |     |     |     |     |     |       |      |
|                               | 1.73±0.09 ab                      | 1.83±0.03 b                 | 1.94±0.02 a          | 1.84±0.07 a          | 1.88±0.04 a        | 1.91±0.02 a         | ***   |      |

The values are presented as LSM ± S.E; the means with letters differ significantly between genotypes (a,b .. p≤0.05; A, B … p≤0.01); * p≤0.05; ** p≤0.01; *** p≤0.001.”

Table 3. The significant association of MYOM1 and ATGL polymorphisms and fatness traits.

| Traits                        | MYOM1 c.2539G>A genotypes | ATGL c.392G>A genotypes | GLM significance |
|-------------------------------|-----------------------------|--------------------------|------------------|
|                               | AA  | AG  | GG  | AA  | AG  | GG  | MYOM1 | ATGL |
| Test daily gain (g/day)        | 863.9±21.4                      | 876.6±8.6                  | 897.5±6.2         | 844.9±16.6 b       | 877.8±10.4 ab      | 898.0±5.9 a        | **    |      |
| Feed: gain ratio (kg/kg)      | 3.02±0.07                       | 2.98±0.03                  | 2.92±0.02         | 3.04±0.05          | 2.96±0.03          | 2.92±0.02          |       |      |
| Age at slaughter (day)         | 179.3±2.9                       | 180.0±1.6                  | 179.1±0.8         | 185.0±2.2 a        | 177.9±1.4 b        | 179.2±0.8 b        |       |      |

The values are presented as LSM ± S.E; the means with letters differ significantly between genotypes (a, b .. p≤0.05; A, B … p≤0.01); * p≤0.05; ** p≤0.01;
Table 4. The significant association of *MYOM1* and *ATGL* polymorphisms and meat quality traits.

| Traits   | MYOM1c.2539G>A genotypes | ATGLc.392G>A genotypes | GLM significance |
|----------|---------------------------|-------------------------|------------------|
|          | AA | AG | GG | AA | AG | GG | MYOM1 | ATLG |
| WE       | 33.9±1.98 | 33.2±0.84 | 34.5±0.66 | 35.6±1.67 a | 36.5±1.06 ab | 33.1±0.61 b | **   |
| *L       | 54.8±1.3 | 54.6±1.0 | 54.1±0.9 | 54.5±1.2 | 54.2±1.1 | 54.3±0.9 |       |
| *a       | 16.6±0.8 | 16.3±0.6 | 16.3±0.6 | 16.6±0.7 | 16.3±0.7 | 16.2±0.6 |       |
| *b       | 3.9±0.7 | 4.0±0.5 | 3.8±0.5 | 4.0±0.6 | 3.4±0.5 | 3.9±0.5 |       |
| pH45_loin| 6.28±0.19 | 6.50±0.09 | 6.37±0.06 | 6.37±0.17 | 6.39±0.10 | 6.42±0.06 |       |
| pH24_loin| 5.57±0.04 | 5.64±0.02 | 5.66±0.01 | 5.65±0.04 | 5.65±0.02 | 5.66±0.01 |       |
| pH45_ham | 6.43±0.14 | 6.54±0.14 | 6.50±0.14 | 6.43±0.15 | 6.55±0.14 | 6.51±0.14 |       |
| pH2_ham  | 6.43±0.15 | 6.54±0.14 | 6.50±0.14 | 5.59±0.09 | 5.57±0.09 | 5.58±0.08 |       |
| IMF      | 1.83±0.18 | 1.73±0.14 | 1.66±0.14 | 1.75±0.17 | 1.67±0.15 | 1.70±0.14 |       |

The values are presented as LSM ± S.E; the means with letters differ significantly between genotypes (a, b .., p≤0.05); WE – water exudation; L* – luminosity, a* – redness, b* – yellowness; pH45 and pH24 – pH measured 45 min and 24 h after slaughter in *longissimus dorsi* (loin) or *semimembranosus* (ham) muscles. IMF – intramuscular fat content.
Figure 1. Allelic Discrimination Plot (TaqMan Genotyper Software v1.4); A – ATGL c.392G>A; B – MYOM1 c.2539G>A; red dots – homozygotes GG; blue dots – homozygotes AA; green dots – heterozygotes G/A; yellow dots – lack of amplification; light blue squares – negative controls.

Figure 2. Genotypes distributions for both analyzed polymorphisms within ATGL and MYOM1 genes.