

Detection of the *rs109815800* and *rs210941459* polymorphisms in the *PLAG1* gene and their effect on growth traits in Limousin cattle

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SUMMARY

The aim of this experiment was to evaluate possible relationships between two SNPs in the *PLAG1* gene and certain cattle traits, such as birth weight, average daily gains between birth and weaning, weaning weight adjusted to 210 days of age, age at first calving, body weight of cows, muscularity, height at hip cross, and chest girth. The study was conducted using 102 Limousin cows. The genotypes were identified by the PCR-RFLP method. The frequencies of the most common alleles and genotypes were as follows: *rs109815800* – allele G 0.523 and genotype GT 0.355; *rs210941459* – allele C 0.584 and genotype CC 0.411. The results of the statistical analysis for both polymorphisms indicate significant differences ($P \leq 0.05$, $P \leq 0.01$) for average daily gains between birth and weaning, weaning weight adjusted to 210 days of age, age at first calving, and body weight of cows. In the case of the *rs210941459* polymorphism, an association with muscularity was shown as well. Therefore, this study can be a first step in increasing knowledge about cattle and help to improve selection of this species to ensure the quality of its growth characteristics.

KEY WORDS: *PLAG1* gene, growth traits, Limousin cattle, gene polymorphism

INTRODUCTION

The purpose of the breeding programme for the Limousin breed in Poland is to preserve or improve its characteristic performance traits (Przysucha and Grodzki, 2007). Features that significantly improve the profitability of production are enhanced, including very good gains, ease of calving, meat performance, appropriate body weight, well-developed bones, and a capacious



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Received: 25.01.2024

Received in revised form: 30.01.2024

Accepted: 12.03.2024

Published online: 13.03.2024

chest. Evaluation of body conformation is one of the elements of assessment of utility value and can be the basis for initial selection of animals, particularly the choice of bulls in a herd of beef cattle (Choroszy et al., 2012). Existing links between production and functional characteristics in selection can make this process more rigorous at the farm level and improve the animals' productivity.

The gene product encoding pleomorphic adenoma 1 (PLAG1) is involved in cell proliferation by directly regulating many target genes, including those encoding various growth factors, such as IGF2 (Van Dyck et al., 2007). *PLAG1* belongs to the highly conserved family of transcription factors containing the zinc finger motif (Juma et al., 2016). The *PLAG1* gene in cattle has been mapped on chromosome 14. It consists of four exons, including two coding ones, and is 2001 base pairs long, which translates into a 499 amino acid protein (<https://www.ensembl.org/index.html>). Genetic research has shown that *PLAG1* regulates cell apoptosis and stops the cell cycle in the G1 phase, and that it is associated with the development of various cancers, such as germ cell adenoma (Zhou et al., 2019). *PLAG1* might be an important candidate gene for potentially influencing the growth and development of domestic animals due to its crucial role in the cell cycle. Furthermore, some studies have shown that *PLAG1* is involved in regulation of milk production, reproductive performance, muscle building, and the body height of livestock (Juma et al., 2016, Fink et al., 2017).

Based on previous work on the connection between the *PLAG1* gene and cattle growth traits, the study aimed to estimate the frequency of genotypes and alleles of two selected polymorphisms, *rs109815800* and *rs210941459*, in the *PLAG1* gene and to determine the potential links between individual genotypes and selected parameters of body conformation of Limousin cattle.

MATERIALS AND METHODS

The study included 102 Limousin cows. It was carried out using material collected in eastern Poland (Podlasie and eastern Mazovia regions). The animals were kept on five commercial farms, all under similar environmental conditions. A similar fattening model was used on all farms. In the spring and summer, the cows were given access to the pasture; in the winter they were fed hay and haylage. The performance value of the cattle was assessed in accordance with Commission Decision 2006/427/EC of 20 June 2006 laying down performance monitoring methods and methods for assessing cattle's genetic value for pure-bred breeding animals of the bovine species (OJ EU L 169 of 22.06. 2006, p. 56). The study assessed the birth weight, average daily gains between birth and weaning, weaning weight adjusted to 210 days of age, and then body weight, height at hip cross, chest girth, and age at first calving.

The body weight of the animals was determined using an electronic scale. After birth, the calves stayed with their mothers for 7–8 months, after which they were weaned. In the period before weaning, the calves used the pasture, took their mother's milk, and received crushed cereal grains (a mixture of barley, oats, and triticale). The cows were kept in free-range buildings during the winter and on the pasture from the beginning of May to the end of October. Reproduction was based on harem mating and artificial insemination. The births of both multiparous and primiparous cows took place from November to March and were very easy, with no need for human intervention.

Data on the cows' performance was obtained from breeding documentation kept at the farm (heifer/cow record) and by the Polish Federation of Cattle Breeders and Milk Producers.

Peripheral whole blood was collected from each subject into vacuum tubes containing K₃EDTA (anticoagulant factor). DNA was isolated using the MasterPure™ Complete DNA and RNA Purification Kit (Lucigen Corporation) according to the protocol provided with the kit. Purified DNA was suspended in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) and stored at 4°C (Sigma Aldrich, St. Louis, MO, USA).

The PCR-RFLP method was used to perform genotyping. Two single nucleotide substitution polymorphisms (SNPs) in the *PLAG1* gene were analysed: *rs109815800* (14: g.23338890G> T) and *rs210941459* (3' gene region). For the *rs210941459* polymorphism, genotyping was performed according to the methodology described by Zhong et al. (2019). For the other polymorphism, a pair of primers was developed using the artificially created restriction site method (ACRS-PCR) to create a cleavage site for the restriction enzyme *Nla*III. A previously designed pair of primers (F: 5'-CACTCAGCAAAAATTTTACTTCC -3' and R: 5'-CTATCATAATCAAAGTTTCAGTCTCAT - 3') was used to obtain an amplification product of 461 base pairs. The temperature profile of the amplification was as follows: initial denaturation 94°C/5 min, then 30 replications of denaturation 94°C/30 s, primer annealing 48°C/40 s, extension 72°C/30 s, and final extension 72°C/5 min.

Based on the genotyping results, the frequencies of genotypes and alleles were counted. The Hardy–Weinberg (HWE) equilibrium, normality of distribution, and genetic indicators of the population, i.e. He gene heterozygosity and the effective number of alleles (*N_e*), were analysed, and polymorphism information content (PIC) was calculated (Botstein et al, 1980). The calculations were performed using PopGene ver. 1.32 (Yeh et al., 2000) and the method of Nei and Roychoudhury (1974).

Statistical analysis of the relationship between the genotypes of *rs109815800* and *rs210941459* polymorphisms and selected cattle performance parameters was carried out. The significance of differences between the means was estimated using the t-test and Fisher's LSD (least significant difference) test ($p \leq 0.05$; $p \leq 0.01$). Pearson's correlations were estimated and tested for pairs of random variables ($p \leq 0.05$; $p \leq 0.01$) using the CORR Sas Enterprise Guide procedure (SAS / STAT 9.4, 2013).

RESULTS

As a result of restriction enzyme cleavage of the PCR products, with lengths of 461 bp and 628 bp for *rs109815800* and *rs210941459*, respectively, the presence of all three genotypes for both polymorphisms was demonstrated. The frequencies of individual genotypes and alleles for the polymorphisms are presented in Table 1. The data in the table show that the frequencies of genotypes and alleles for both SNPs are very similar. The heterozygote parameters *H_e* are 0.499 and 0.465, which indicates that in the polymorphic locus, a random individual many have any two different alleles, which in practice means that the probability of heterozygous occurrence in the population is over 40%. The PIC value was calculated for both loci. The PIC values were 0.374 and 0.368, which is a high value for this index (PIC value > 0.50, Nei and Roychoudhury, 1974), indicating high genetic diversity.

Table 1.
Genotype and allele frequencies

Polymorphism	N	Genotype frequencies		Allele frequencies		χ^2 (HWE)	He	Ne	PIC
<i>rs109815800</i>	37	<i>GG</i>	0,345	<i>G</i>	0,523	8,885**	0.499	1,996	0,374
	38	<i>GT</i>	0,355						
	32	<i>TT</i>	0,300						
<i>rs210941459</i>	26	<i>TT</i>	0,243	<i>T</i>	0,416	8,891**	0.465	1.869	0,368
	37	<i>TC</i>	0,346						
	44	<i>CC</i>	0,411						

** – significant difference between alleles at $p < 0.01$

N – number of cows; He - gene heterozygosity, Ne - effective allele numbers, PIC - polymorphism information content

The results of the analysis of the connection between the two polymorphisms (*rs109815800* and *rs210941459*) and the meat performance traits of Limousin cattle are shown in Table 2.

The analysis of the connections between the *rs109815800* and *rs210941459* genotypes and the body weight of Limousin cattle revealed convergent relationships, i.e. the heterozygous genotypes of both polymorphisms were associated with the highest body weight; differences were significant at $P \leq 0.05$. The birth weight of calves was highest in heterozygous individuals *rs109815800* (31.13 kg), while in the case of the *rs210941459* polymorphism, the highest weight was achieved by calves with the *CC* homozygous genotype (31.07 kg). Statistically significant differences ($P \leq 0.05$, $P \leq 0.05$) were observed between cows with different genotypes for daily gains and body weight standardized for 210 days of age in both polymorphisms. Average daily gains (1063.47 kg) were highest for homozygous *GG* individuals (*rs109815800*) and slightly lower gains (1060.46 kg) in individuals heterozygous for SNP *rs210941459*. Other features, such as muscularity and height at hip cross, were most favourable for the heterozygous individuals with the *rs109815800* polymorphism. Significant differences ($P \leq 0.05$) associated with muscularity were observed between genotypes of animals in the case of both polymorphisms. Individuals with the *GT* genotype had significantly better muscularity (16.55 points) than the other genotypes. The chest circumference of the animals was most favourable in animals with the *GG* *rs109815800* genotype (193.52 cm). Cows with the *TC* genotype (*rs210941459*) were slightly inferior in terms of this trait (the *TT* genotype determined the lowest value). There were no statistically significant differences in chest circumference between individuals of different genotypes. The first calving was latest in cows with the *TT* genotype (*rs210941459*) (979.15 days) and earliest in those with the *CC* genotype (923.2 days). Similarly, *TT* homozygous individuals (*rs109815800*) had the latest first calving age (969.55 days). These relationships were statistically confirmed ($P \leq 0.01$) between the genotypes of animals with both polymorphisms.

Table 2.

Analysis of the relationship between polymorphisms in the *PLAG1* gene and conformation traits of Limousin cattle (mean \pm SD)

SNP	Traits	Genotype		
		GG	GT	TT
<i>rs109815800</i>	BWT	31.05 \pm 1.81	31.13 \pm 2.23	30.81 \pm 1.06
	ADG	1063.47 ^b \pm 47.14	969.68 ^a \pm 14.53	889.41 ^c \pm 33.54
	WWT210	254.39 ^B \pm 9.97	234.2 ^A \pm 4.55	216.94 ^C \pm 7.13
	AFC	924.92 ^A \pm 88.41	922.37 ^A \pm 118.66	969.66 ^B \pm 162.38
	BW	555 ^b \pm 26.9	556 ^a \pm 30.09	560.93 ^c \pm 21.48
	M	14.29 \pm 4.71	16.65 ^a \pm 3.02	14.3 ^b \pm 4.76
	HHC	132.92 \pm 1.96	133.25 \pm 1.72	132.13 \pm 1.86
	CHG	193.52 \pm 3.07	193.34 \pm 2.15	192.32 \pm 2.61
<i>rs210941459</i>		TT	TC	CC
	BWT	30.85 \pm 1.12	31.05 \pm 2.10	31.07 \pm 2.1
	ADG	878.88 ^c \pm 27.69	1060.46 ^b \pm 47.14	964.95 ^a \pm 18.3
	WWT210	215.4 ^C \pm 5,78	254.39 ^B \pm 9.97	230.24 ^A \pm 8.22
	AFC	979.15 ^B \pm 156.34	924.92 ^A \pm 88.41	923.2A \pm 128.94
	BW	556.67 ^b \pm 21.55	555.00 ^b \pm 26.9	568.77 ^a \pm 26.99
	M	13.39 ^c \pm 5.02	14.29 ^b \pm 4.71	16.81 ^a \pm 2.79
	HHC	132.04 \pm 2.01	132.92 \pm 4.96	133.13 \pm 1.65
CHG	192.12 \pm 2.83	193.42 \pm 2.87	193.32 \pm 2.01	

BWT – birth weight; ADG – average daily gains between birth and weaning; WWT210 – weaning weight adjusted to 210 days of age; AFC - age at first calving; BW– body weight of cows; M – muscularity; HHC - height at hip cross; CHG - chest girth

a, b – values in rows with lowercase letters differ significantly at $P \leq 0.05$.

A, B – values in rows with a capital letter differ significantly at $P \leq 0.01$.

Values of phenotypic correlation coefficients between features, together with their significance level, are shown in Table 3. Highly significant correlations ($p \leq 0.01$) were recorded between the musculature and weight of the cow; height at hip cross and muscles; and chest circumference and age at first calving. Highly significant correlations ($p \leq 0.01$) were also shown for WWT210 and BWT and for ADG and WWT210. Significant negative correlations ($p \leq 0.05$) were found between muscularity and age at first calving; height at hip cross and age at first calving; and BWT and age at first calving. The remaining correlation coefficients were statistically insignificant.

Table 3.

Pearson's correlation values for growth parameters of Limousin cows

Traits	AFC	BW	M	HHC	CHG	BWT	WWT210	ADG
AFC	1	-0.13838 ^{ns}	-0.27188*	-0.22500*	-0.35107**	-0.21184*	-0.12914 ^{ns}	-0.12870 ^{ns}
BW		1	0.70414**	0.28962 ^{ns}	0.41843**	0.23384 ^{ns}	-0.09395 ^{ns}	-0.07327 ^{ns}
M			1	0.50800**	0.61612**	-0.07151 ^{ns}	0.01866 ^{ns}	0.09266 ^{ns}
HHC				1	0.84222**	-0.16809 ^{ns}	0.19409 ^{ns}	0.17611 ^{ns}
CHG					1	-0.02269 ^{ns}	0.14000 ^{ns}	0.17740 ^{ns}
BWT						1	0.98235**	0.03849 ^{ns}
WWT210							1	0.98235**
ADG								1

r – Pearson's correlation: ** p ≤ 0.01; * p ≤ 0.05; ns = p > 0.05

AFC - age at first calving; BW – body weight of cows; M – muscularity; HHC - height at hip cross; CHG - chest girth; BWT – birth weight; WWT210 – weaning weight adjusted to 210 days of age; ADG – average daily gains between birth and weaning

DISCUSSION

Livestock breeding programmes are based primarily on the phenotypic value of the desired animal performance traits and appropriate selection of animals for mating. To improve the efficiency of selection, the classic method is increasingly supported by the use of molecular markers associated with selected features. The gradual development of molecular techniques enables a better understanding of the genetic basis of performance traits, and thus for better use of the available information about molecular markers in the design of breeding programmes. Therefore, marker-assisted selection (MAS) and assessment of genomic breeding value (GBV) are becoming increasingly popular.

The *rs109815800* and *rs210941459* polymorphisms have thus far only been studied in Chinese cattle herds (Hou et al., 2020; Zhong et al., 2019). Hou et al. (2020) studied many different breeds of cattle from various parts of China. According to their geographic distribution they were assigned to northern, central and southern groups, and a control group represented by Angus and Holstein cattle was added. In the Chinese cattle breeds, the *G* allele was significantly more frequent, while in the control group, the *T* allele was the most prevalent (0.523). Zhong et al. (2019) estimated the allele frequencies for the *rs210941459* polymorphism in several Chinese cattle breeds and showed that the *T* allele was much more common (0.663–0.744); the *C* allele had a higher frequency in only one breed. In our study, however, the frequency of alleles was fairly even, with prevalence of the *C* allele (0.584).

Polymorphism in the *PLAG1* gene has been investigated by various authors, mainly with respect to the growth characteristics of meat cattle. In the present study, two polymorphisms were analysed: *rs109815800* and *rs210941459*. The same polymorphisms were studied by Hou et al. (2020) and by Zhong et al. (2019). The authors cited conducted studies on various breeds of

Chinese cattle and showed a correlation between the SNPs and the body height of the animals in both cases. Zhong et al. (2019) also showed an association with features such as chest girth and height at hip cross. Putra et al. (2021) analysed seven SNPs mapped in exon 2 of the *PLAG1* gene, and only one of them (c.957A> C) proved to be polymorphic, but unfortunately they found no statistically significant correlation with any of the traits studied (birth weight, weaning weight at 205 days of age, yearling weight at 365 days of age, yearling weight at 730 days of age, and average daily gain). Other researchers (Xu et al., 2018, Zhou et al., 2019) focused on an insertion-deletion polymorphism (19-bp Indel; *rs523753416*) in cattle herds of Chinese breeds and demonstrated an association with body height, height at sacrum, chest depth, rump length, and heart girth.

The role of the *PLAG1* gene in the growth of organisms has been confirmed by a series of genome-wide association studies (GWAS) in both humans and cattle. SNPs mapped in the chromosome fragment containing *PLAG1* and its adjacent genes were shown to manifest the strongest correlation with adult human height (Caucasian and Asian populations) (Juma et al., 2016). Pig and horse studies have also shown that different *PLAG1* variants may affect growth traits, suggesting that this particular role of *PLAG1* is common to mammals (Rubin et al., 2012, Metzger et al., 2013). It should also be emphasized that *PLAG1* was found to be the most likely candidate for a causative gene of growth differences in cattle, if only because of its location on chromosome 14 near other genes such as *RPS20*, *MOS*, *RDHE2*, *SDR16C6* and *PENK*, which have established links with growth (Juma et al., 2016).

There have been few literature studies regarding the *rs109815800* and *rs210941459* polymorphisms in the bovine *PLAG1* gene and the parameters of meat performance analysed in the study. Assessment of the conformation of beef cattle, taking into account the animal's type and structure, is an important tool in breeding work. It provides information about the animal's structure and its production predispositions and enables selection of the type of production, which is important in breeding. Negative and statistically significant correlations between age at first calving and muscularity and between height at hip cross and BWT confirm the physiological and developmental link. The trait of muscularity in beef cattle determines their meat productivity, i.e. carcass quality, share of valuable cuts, and meat content in the carcass. From an economic point of view, the aim should be to lower the age of calving. However, calving of a cow which has not yet reached adult size and weight will make it less tall and less muscular. This trend manifests itself in the correlation coefficients between body weight and height at hip cross and between muscularity and body weight.

Body conformation results are used as criteria to infer carcass quality (Shiotsuki et al., 2009). To select the best specimens as parents of the next generation, it is necessary to conduct an assessment of the animal's conformation. Assessment of body conformation in breeding work provides a great deal of information about the animal's body structure and its production predispositions.

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