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RESEARCH ARTICLE

The Polymorphism in the *IGF1R* Gene is Associated with Body Weight and Average Daily Weight Gain in Pomeranian Coarsewool Ewes

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ABSTRACT

The aim of this study was to investigate the association of five single nucleotide polymorphisms (SNPs) in *GHR* (growth hormone receptor), *LEP* (leptin), *IGF1* (insulin-like growth factor 1), and *IGF1R* (insulin-like growth factor 1 receptor) genes with body weight at day 1, 33 and 90 of age and average weight gain at 1-33, 33-90 and 1-90 days of age in Pomeranian Coarsewool sheep. Blood samples were collected from 100 ewes derived from the only flock of this breed in Poland, and after DNA isolation, five selected loci were genotyped with use of appropriately designed PCR-RFLP assays. The g.122A>G in the *GHR* gene and the g.251G>A in the *LEP* gene were monomorphic, so only the effects of g.367G>T in the *LEP* gene, g.271C>T in *IGF1* gene, and g.195C>T in *IGF1R* gene were investigated. The statistical analysis showed no association of the g.367G>A in *LEP* gene and g.271C >T in *IGF1* gene with the selected growth parameters. In contrast, the g.195C>T polymorphism in *IGF1R* gene was significantly associated ($P<0.001$) with body weight and average daily weight gain. The *TT* genotype was linked to the highest values, while the *CC* genotype was linked to the lowest values, of the all analyzed traits. Thus, this study indicated the g.195C>T SNP as a potential genetic marker for growth traits. Nevertheless that effect should be investigated in other sheep breeds in order to confirm if that SNP can be used for marker-assisted selection.

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INTRODUCTION

Lamb weight and average weight gains are pivotal traits in sheep breeding. They are affected by both environmental and genetic factors (Akhtar *et al.*, 2012). Numerous genes are involved in the process of growth. The most important among them seem to be those participating in the somatotropic axis - growth hormone (*GH*), insulin-like growth factor 1 (*IGF1*), leptin (*LEP*), and genes coding for their receptors (Maksymiec and Mikolajczyk, 2012; Du *et al.*, 2013; Bahrami *et al.*, 2013).

The growth hormone is the main constituent of the somatotropic axis and plays crucial role in the postnatal growth and metabolism regulation. It affects target tissues directly by binding to growth hormone receptors (GHR) (Tuggle and Trenkle, 1996). Additionally, GH affects indirectly by controlling the secretion of other hormones including IGF1, which interacts with insulin-like growth factor 1 receptors (IGF1R) in target tissues (Jones and Clemmons, 1995).

Another very important hormone is leptin which is synthesized by the adipocyte tissue. It plays pivotal role in regulation of feed intake and body weight in many species including ruminants. It was reported that leptin upregulates the level of GH in blood plasma (Wójcik-Gładysz *et al.*, 2010).

As far as the biological function of the *GHR*, *LEP*, *IGF1*, and *IGF1R* genes is considered, they seem to be good targets for research aimed at indication of genetic markers for growth traits such as body weight or average daily weight gain in farm animals. The aim of this study was to investigate the single nucleotide polymorphisms (SNPs) in those genes and to test its influence on selected growth traits i.e. body weight at day 1, 33 and 90 of age and average weight gain at 1-33, 33-90 and 1-90 days of age in Pomeranian Coarsewool sheep.

MATERIALS AND METHODS

Animals: The study was performed in the only flock in Poland of Pomeranian Coarsewool Sheep, also known as

Table 1: Primer sequences and restriction enzymes used for the PCR-RFLP genotyping of the selected single nucleotide polymorphisms

Gene	SNP	Primer sequences	Tm	Length	RE	Accession
GHR	g.122A>G	F-CCAGCAGGAAATGTGGTCCT	60°C	247	*Bgl*I	AY292283
exon 10	synonymous	R-CGGCTGTAGTGGTAAGGCTT				
LEP	g.251G>A	F-GCATAGCAGTCCGTCTCCTC	60°C	340	*Nci*I	U84247
exon 3	p.R84Q	R-GCCGCAACATGTCCTGTAGA				
LEP	g.367G>T	F-GCATAGCAGTCCGTCTCCTC	60°C	340	*Msl*I	U84247
exon 3	p.V123L	R-GCCGCAACATGTCCTGTAGA				
IGF1	g.271C>T	F-AGCAGGTGAAGATGCCAGTC	60°C	272	*Ava*I	X69473
exon 3	synonymous	R-TGAGGAATCTCGGAGGCTGA				
IGF1R	g.195C>T	F-TCCCAAGTGGAGGTGAGTCT	59.5°C	206	*Rsa*I	KJ140106
intron 12*	intronic	R-ATAAGCCAGCTCCTGCACAC				

SNP: single nucleotide polymorphism; Tm: annealing temperature; RE: restriction enzyme; *numbered in accordance with human and bovine sequences

Table 2: Genotypic and allelic frequencies of the selected single nucleotide polymorphisms in Pomeranian Coarsewool sheep (n=100)

SNP	Genotype			Allele	
GHR exon 10	*AA*	*AG*	*GG*	*A*	*G*
g.122A>G	1.00	–	–	1.00	–
LEP exon 3	*GG*	*GA*	*AA*	*G*	*A*
g.251G>A	1.00	–	–	1.00	–
LEP exon 3	*GG*	*GT*	*TT*	*G*	*T*
g.367G>T	0.9	0.1	–	0.95	0.05
IGF1 exon 3	*CC*	*CT*	*TT*	*C*	*T*
g.271C>T	0.05	0.31	0.64	0.205	0.795
IGF1R intron 12	*CC*	*CT*	*TT*	*C*	*T*
g.195C>T	0.14	0.39	0.47	0.335	0.665

Rough-coated Pomeranian Landrace sheep in Poland (only ewes; n=100), which were imported to the area of West Pomerania in 2004. The animals were kept on organic farm situated near the Łąki Skoszewskie area belonging to the Natura 2000 - the program of protection of threatened habitats and species across Europe.

Growth traits: The animals were weighed 3 times: just after birth at their 1st day of life, and also at 33rd and 90th day of life. Additionally, average daily weight gain was estimated for the period from 1st to 33rd, 33rd to 90th and 1st to 90th day of age.

DNA isolation and genotyping: Whole peripheral blood was collected in test tubes containing an anticoagulant (K_3EDTA) from the jugular vein of adult ewes after their first lambing. The DNA was isolated from 300 µl of blood using MasterPure™ kit (Epicentre Biotechnologies, Madison, WI). The genotyping of the selected SNPs was performed by using the appropriate PCR-RFLP approaches (Table 1). The PCR mixture contained ~50 ng of genomic DNA, 20 pmol of each primer, 1xPCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 units of Taq-polymerase and filled up to 20 µl with deionized water. The following cycles were applied: denaturation at 94ºC/5 min, followed by 33 cycles at 94ºC/50 sec, primer annealing at 59.5ºC or 60ºC for 60 sec, DNA fragments synthesis at 72ºC/50 sec, and final synthesis at 72ºC/7 min. The PCR products were digested with 5 units of an appropriate restriction enzyme at 37ºC. The DNA restriction fragments were separated in 2% agarose gel and stained with ethidium bromide. The results of electrophoretic separations were visualized under UV light and the gel photographs were archived. The primer sequences and all the necessary data are given in Table 1.

Statistical analysis: The association of particular SNP with selected growth traits was tested by using ANOVA with an inclusion of the birth type (single or twin) of an ewe and genotype as a fixed effect. Bonferroni test was used for multiple comparisons. The analyses were performed with use of Statistica 10 package (StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Five SNPs were genotyped with use of the PCR-RFLP approaches among which the g.251G>A in the *LEP* gene and the g.122A>G in the *GHR* gene were monomorphic. The genotype and allele frequencies for all analyzed SNPs are given in Table 2.

The g.122A>G located in the exon 3 of the *GHR* gene was reported and investigated in several studies. Bahrami *et al.* (2013) indicated all analyzed animals as homozygotes for that locus, but they did not specify whether they were of *GG* or *AA* genotype, while all sheep were *AA* homozygotes in this study. In contrast, Pariset *et al.* (2006) indicated the presence of rare *G* allele in 8 sheep breeds with frequency ranging from 0.05 in Karagouniko breed to 0.409 in Akkaraman breed. Additionally, they reported the frequency of *G* allele (f=0.15) in Żelaźnieńska sheep, which is Polish indigenous breed.

All the population investigated in this study was *GG* homozygotic for the g.251G>A in the *LEP* gene. Pariset *et al.* (2006) obtained similar results in 7 different sheep breeds – they designated that SNP as g.314A>G with reference to the bovine *LEP* sequence (U43943). Nevertheless, they also reported the presence of rare *A* allele (f=0.139) in Welsh Mountain ewes. In case of g.367G>T in the *LEP* gene, we indicated the presence of both *G* and *T* (f=0.05) alleles combined into *GG* and *GT* genotypes. In contrast, Reicher *et al.* (2011) found the *TT* genotype in Assaf and Improved Awassi sheep with frequency equal to 0.08 in both breeds, while *T* allele frequencies were 0.26 and 0.21, respectively.

The analysis of the g.271C>T in the *IGF1* gene revealed the presence of both alleles and 3 possible genotypes: *CC*, *CT* and *TT*. The *T* allele was predominant in Pomeranian Coarsewool sheep analyzed in this study (f=0.795) and similar observation was reported by Scatà *et al.* (2010) in Gentile di Puglia (f=0.613) and Sarda (f=0.545), but not in Altamurana sheep (f=0.431). In turn, Gholibeikifard *et al.* (2013) indicated a very low frequency of the *T* allele (f=0.05) in Baluchi sheep; they did not find the *TT* genotype as opposed to this study.

The genotyping of the g.195C>T substitution within intron 12 of the *IGF1R* gene showed the presence of both alleles, and the *T* allele was predominant (f=0.665). Three

Table 3: Association of g.195C>T polymorphism in *IGF1R* gene with body weight and average weight gain in Pomeranian Coarsewool ewes

Genotype	n	Weight (kg)			Average weight gain (g/day)		
		1	33	90	1-33	33-90	1-90
CC	14	2.87±0.15[a]	6.95±0.31[a]	19.01±0.39[a]	127±8[a]	212±6[a]	181±3[a]
CT	39	2.97±0.21[a]	7.24±0.30[b]	19.60±0.53[b]	133±8[a]	217±9[a]	187±5[b]
TT	47	3.17±0.17[b]	7.94±0.39[c]	21.51±0.66[c]	149±10[b]	238±11[b]	206±7[c]
Total	100	3.05±0.22	7.53±0.53	20.42±1.20	140±13	226±15	195±12

The 1, 33 and 90 correspond to the day of age when the ewes were weighed. Different superscript letters within columns indicate statistically significant differences (P≤0.05).

genotypes were observed at this locus and their frequencies are given in Table 2.

The next step was to investigate possible influence of particular genotypes on growth traits in Pomeranian Coarsewool sheep. As the g.251G>A in the *LEP* gene and g.122A>G in the *GHR* gene were monomorphic, only the effects of g.367G>A in *LEP* gene, g.271C>T in *IGF1* gene and g.195C>T in *IGF1R* gene on the selected traits were investigated. The statistical analysis indicated that the g.367G>A in *LEP* gene and g.271C>T in *IGF1* gene were not linked to any of growth parameters. In contrast, the g.195C>T polymorphism in *IGF1R* gene was significantly associated (P<0.001) with body weight at 1st, 33rd and 90th day of age and also with average daily weight gain in all analyzed periods (Table 3). The *TT* genotype was linked to the highest values, while the *CC* homozygotic ewes were characterized by the lowest values of the all analyzed traits. The differences between *CC* and *TT* carriers were the most noticeable in case of body weight at 1st day and average daily weight gain from 1st to 33rd day, since the *TT* animals were characterized by 10 and 17% higher values of these traits, respectively.

IGF1R is a pivotal component of the IGF1 signaling pathway and is activated by binding of either the IGF1 or insulin (Adams *et al.*, 2000; Byun *et al.*, 2012). Hence, a polymorphism in the *IGF1R* may alter the function of the receptor by changing its ability to proper binding of the IGF1. Since the IGF1 is known to be strictly involved in the control of growth and organ size in mammals, mutations in the *IGF1R* may lead to violation of IGF1 signaling pathway, thus affecting growth traits (Adams *et al.*, 2000).

Up to date only a little is known about structure of ovine *IGF1R* gene, as well as about its variation and functional implications of particular polymorphisms. Herein we investigated the g.195C>T substitution in intron 12 of that gene. While this polymorphism does not cause an amino acid change, it may nevertheless be in linkage disequilibrium with some other functional genetic variant, within either the coding or regulatory region of the *IGF1R* gene, which is responsible for changes in growth-related traits. The finding of an association between polymorphism in the *IGF1R* gene and growth traits is in agreement with numerous reports in other species. The effect of polymorphism in *IGF1R* gene on body weight was showed in beef cattle (De la Rosa Reyna *et al*., 2010), pig (Wang *et al*., 2005), yak (Liang *et al*., 2010), chicken (Lei *et al*., 2008) and Japanese quail (Moe *et al*., 2007). Moreover, the influence of *IGF1R* gene variation on average daily weight gain was also investigated and confirmed in many species such as Egyptian buffalo (El-Magd *et al*., 2013), beef cattle (De la Rosa Reyna *et al*., 2010), chicken (Lei *et al*., 2008) and Japanese quail (Moe *et al*., 2007). Additionally, Hoopes *et al*. (2012) reported the SNP linked to tiny size in dogs that changes a highly conserved arginine at amino acid 204 to histidine in *IGF1R*. The *IGF1R* gene is strong functional candidate for growth traits in many species based on the recent research, and also in sheep as reported in this study.

Conclusion: This study showed significant association polymorphism within the gene coding for insulin-like growth factor 1 receptor with body weight and average weight gain in Pomeranian Coarsewool ewes, and thus indicated the g.195C>T SNP as a potential genetic marker for growth traits. Nevertheless, that effect should be investigated in other sheep breeds in order to confirm if that SNP can be used for marker-assisted selection.

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