TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2022, vol. 7, No 1(12): 44–48

POLYMORPHISM OF ABCG2 GENE AND ITS EFFECTS ON LITTER SIZE AND MILK PRODUCTION OF SYNTHETIC POPULATION BULGARIAN MILK EWES

Milena Bozhilova-Sakova*[1], Ivona Dimitrova[2], Tanya Ivanova[1], Vesselin Koutev[2], Maya Ignatova[1]

[1]Agricultural Academy, Institute of Animal Science, Kostinbrod, Bulgaria
[2]University of Forestry, Sofia, Bulgaria
E-mail: bojilova_milena@abv.bg

ABSTRACT

The aim of present study was to investigate polymorphism of ABCG2 (ATP-binding cassette sub-family G member 2) gene and its effect on litter size and milk production. The experiment included 30 ewes of Synthetic Population Bulgarian Milk (SPBM) breed from Institute of Animal Science – Kostinbrod. For genotyping of ABCG2 locus it was used only PCR amplification using specific set of primers. As a result two alleles (*D* and *I*) and two genotypes (*ID* and *DD*) were established in studied sheep of SPBM breed for locus ABCG2. The frequency of allele *D* was 0.68 and the frequency of allele *I* was 0.32. The genotype *ID* was with frequency 0.63 and genotype *DD* – with 0.3. In this experiment was studied the relationship between different genotype variants of the tested locus and litter size and milk yield of ewes from Synthetic Population Bulgarian Milk. The comparative analysis was performed using the statistical method ANOVA but no statistically significant differences were observed.

Key words: SPBM sheep breed, ABCG2 gene, polymorphism, milk production, litter size.

Introduction

Bulgaria is one of the countries in EU where the sheep farming is traditional and intensive branch because environmental and climate conditions are quite suitable. This sector ensures a livelihood for part of the rural population. In the last two decades, research on candidate genes that can be used as markers to influence various productive characteristics in sheep has developed extremely rapidly (Gutiérrez-Gil et al., 2014; Mishra et al., 2014; Xu and Li, 2017; Ibrahim et al, 2019). Identification of allele variants of different genes would contribute to management of conventional selection programmes and improving of desirable traits of sheep (Tohidi et al., 2013; Gebreselassie et al., 2020).

The membrane-associated protein encoded by the ABCG2 gene is a member of the superfamily of ATP-linked cassette (ABC) transporters, whose function is related to the transport of various molecules across cell membranes (Higgins, 1992; Silanikove et al., 2014). ABCG2 gene have been selected as a candidate gene influencing milk production traits in sheep based on its function (Árnyasi et al., 2013). The ABCG2 gene is located on chromosome 6 of the genome of domestic sheep (*Ovis aries)* and it is composed of 20 exons separated by 19 introns. It is expressed in certain tissues, including the mammary gland, with expression levels increasing during lactation in some farm animals (Al-Mamun et al., 2015). A mutation that is a 35 bp insertion/deletion (c.683-80_46del) has been identified in the sheep ABCG2 gene (Árnyasi et al., 2013; Oner al., 2014). Hofmannová et al., (2018) found polymorphism in intron 5 of the ABCG2 gene in sheep of the Lacoon and East Frisian breeds grown in the Czech Republic. This mutation in the non-coding region cannot cause changes in the amino acid sequence. But it is known that introns can act as carriers of transcriptional regulatory elements. They can also be a source of non-coding RNA and participate in alternative splicing

(Árnyasi et al., 2013; Hofmannová et al., 2018). In this study, the c.683-80_46del mutation in intron 5 of the ABCG2 gene had an effect on the number of somatic cells in a dairy sheep population.

ABCG2 gene is of interest because of its association with sheep milk production, but the number of studies in this species is very limited (Gutiérrez-Gil et al., 2014).

The aim of the present study was to investigate polymorphisms of ABCG2 gene and its potential effects on litter size and milk production of ewes of Synthetic Population Bulgarian Milk (SPBM) breed.

Materials and methods

This study was conducted in the Laboratory of Genetics of the Faculty of Agriculture, University of Forestry – Sofia, Bulgaria. There were tested 30 ewes of Synthetic Population Bulgarian Milk breed from Institute of Animal Science – Kostinbrod. Blood samples were collected from *v. jugularis* in vacuum tubes with EDTA.

Genomic DNA was obtained by manual commercial kit for DNA purification from whole blood according to the manufacturer's instruction (Illustra Blood GenomicPrep DNA Purification Kit, *GE Healthcare*). DNA quality and quantity were determined using spectrophotometer Biodrop and agarose electrophoresis on 1% agarose gel (Bioline) and 1x TAE buffer (Jena Bioscience).

The PCR reactions were carried out in total volumes of 10 µl, containing 40 ng DNA template, 0.2 µl dd-H_2O, 20 pM of each primer and 5 µl of 2×(1.5 mM $MgCl_2$) MyTaq TM HS Red Mix 2x (Bioline). The primer set (F: 5'-GCCTCTTCTCCCATACGTC -3' and R: 5'- AAACCAGTT-GTGGGCTCATC -3') used for the amplification is according to Árnyasi et al. (2013) and produced 232 bp (with deletion) or 267 bp (no deletion) size of PCR product.

PCR reactions were accomplished by thermocycler QB-96 (Quanta Biotech) under the following conditions: primary denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52.6°C for 65 s, elongation at 72°C for 1 min. The reaction was completed by final extension at 72°C for 10 min.

The genotypes for ABCG2 gene were determined only by polymerase chain reaction and specific set of primers who separate the fragments with presence or absence of mutation (deletion/insertion). The fragment sizes were identified using Ready-to-Use DNA Ladder, 50 bp (Thermo) on 2.5% agarose (Bioline) gel and stained by RedGel Nucleic Acid Stain (Biotium) and visualized under UV light.

Obtained data was analyzed by statistical software Statgraphics Centurion XVIII.

Results and Discussion

After PCR reaction and agarose electrophoresis according to Oner et al. (2014) two alleles (*D* and *I*) and two genotypes (*ID* and *DD*) for locus ABCG2 were revealed. The length of the PCR products with deletion was 232 bp (allele *D*) and without deletion it was 267 bp (allele *I*). Allele *D* was with frequency 0.68 and allele *I* with 0.32. Genotype *ID* was with frequency 0.63 and genotype *DD* with 0.37. The values of the expected heterozygosity exceed the observed one, which indicates the presence of a heterozygous deficit in the studied group of animals (Table 1).

Table 1: Allele and genotype frequencies

Locus	n	Allele frequency		Genotype frequency			Ho	He	p
ABCG2	30	**I**	**D**	**II**	**ID**	**DD**	0.435	0.633	P›0.01
		0.32	0.68	0.00	0.63	0.37			

It has been studied in this work the relationship between different genotype variants of the tested locus and both litter size and milk yield of 30 ewes from Synthetic Population Bulgarian Milk. A comparative analysis of litter size of SPBM sheep with genotypic variants *ID* and *DD* of the ABCG2 gene was performed using the statistical method ANOVA (Tables 2 and 3).

Table 2: Summary Statistics for Litter size for ABCG2 gene

Locus ABCG2	Average	Standard deviation	Coeff. of variation
DD	1,267	0,296987	23,4402%
ID	1,26263	0,29758	23,5682%
Total	1,26414	0,292031	23,1012%

The analysis showed that there was no statistically significant difference in litter size between heterozygous individuals with genotype ID and those with homozygous genotype DD by ABCG2 gene at a significance level of 5% (Table 3 and Figure 1).

Table 3: Multiple Range Tests for Litter size for ABCG2 gene. Method: 95.0% LSD

ABCG2 gene	Mean	Homogeneous Groups	Contrast	Sig.	Difference	+/- Limits
ID	1,26263	x	DD – ID		0,00436842	0,238385
DD	1,267	x				

Table 3 showed the multiple range test to determine if there was statistically significant difference between mean values of litter size in ABCG2 gene. There were no statistically significant differences between any pair of means at the 95.0% confidence level.

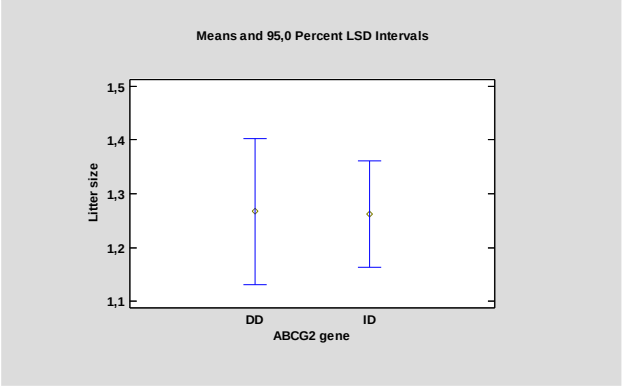

Figure 1: Mean values of Litter size at 95% LSD intervals for ABCG2 gene

Figure 1 showed in the Multiple Range Tests, intervals used to determine if there was a statistically significant difference between the mean values. In our case intervals were not different.

Other economically important trait in sheep is the milk productivity, which is a reason to search an association between milk production during lactation of SPBM ewes and genotype variants *ID* and *DD* of the ABCG2 gene using the statistical method ANOVA (Tables 4, 5).

Table 4: Summary Statistics for Milk Production of lactation for ABCG2 gene

Locus ABCG2	Average	Standard deviation	Coeff. of variation
DD	95,1	10,9334	11,4968%
ID	93,9176	8,69772	9,261%
Total	94,3556	9,39531	9,95734%

The analysis showed that there was no statistically significant difference in milk production between heterozygous individuals with genotype *ID* and those with homozygous genotype *DD* by ABCG2 gene at a significance level of 5% (Table 5 and Figure 2).

Table 5: Multiple Range Tests for Milk production for ABCG2 gene. Method: 95.0% LSD

ABCG2 gene	Mean	Homogeneous Groups	Contrast	Sig.	Difference	+/- Limits
ID	93,9176	x	DD – ID		1,18235	7,84913
DD	95,1	x				

Table 5 showed the Multiple Range Tests to determine if there was statistically significant differences between mean values of milk production. There was no statistically significant difference between mean milk production and different genotypes of ABCG2 gene with 5% significance level.

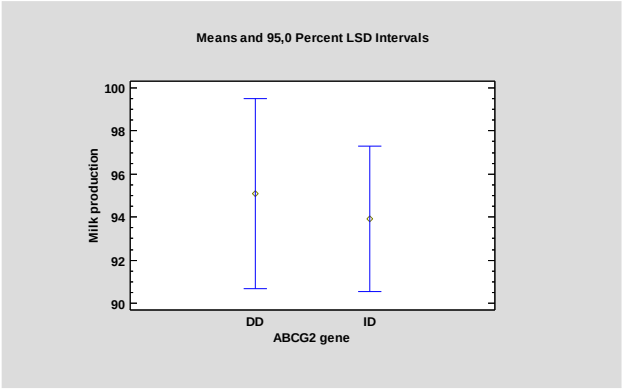

Figure 2: Mean values of Milk production at 95% LSD intervals for ABCG2 gene

Figure 2 showed the Multiple Range Tests intervals used to determine if there was statistically significant difference between mean values of the different genotypes. In our experiment intervals were not statistically different.

In ABCG2 gene were found two alleles with a predominant frequency of allele D (0.68) associated with deletion of 35 bp fragment. In our previous study which included 96 animals from three merino breed – Askanian, Caucasian and Karnobat (Dimitrova et al., 2019) the frequency of allele D was in the range of 0.61 – 0.76. In SPBM ewes two genotypes were identified – ID with the frequency 0.63 and DD with 0.37. In the same study, the three possible genotypes were identified in all three studied breeds, with the highest frequency of the genotype *DD* (from 0.43 to 0.68). Oner et al. (2014) also reported higher frequency of allele D (0.60). They identified three genotypes with preponderance of DD genotype. On the contrary, Árnyasi et al. (2013) announced that the allele I and heterozygous genotype *ID* had higher frequencies in their study. Hofmannová et al. (2018) identified all three genotypes. In breed Lacaune were established predominantly both allele D with deletion (0.69) and genotype DD (0.52), whereas in East Friesian chiefly occurred allele I without deletion (0.78) and genotype II (0.64).

Conclusion

Two alleles (*D* and *I*) and two genotypes (*ID* and *DD*) were established in studied sheep of SPBM breed for locus ABCG2. After comparative analysis of genotypes and milk productivity and litter size no statistically significant differences were found.

Acknowledgements

This research was part of the project КП-06-Н 26-9/18.12.2018 (2018–2021) "Investigation of DNA markers associated with production in sheep breeds reared in Bulgaria" financed by NSF – the Ministry of Education and Science, Republic of Bulgaria

References

1. Al-Mamun, H. A., P. Kwan, S.A. ClarkFerdosi, M.H. Tellam, and C. Gondro. (2015). *Genome-wide association study of body weight in Australian Merino sheep reveals an orthologous region on OAR6 to human and bovine genomic regions affecting height and weight*. Genet Sel Evol, 47, 47–66.
2. Árnyasi, M., I. Komlósi, M.P. Kent, L. Czeglédi, G. Gulyás, and A. Jávor. (2013*). Investigation of polymorphisms and association of the ABCG2 gene with milk production traits in sheep*. Livestock Science, 154, 64-68.
3. Dimitrova, I., M. Bozhilova-Sakova, M. Ignatova, V. Koutev and K. Genova. (2019). *Investigation of ABCG2 gene in three fine fleece sheep breeds in Bulgaria*. in: Proceedings of International Congress on Domestic Animal Breeding, Genetics and Husbandry 2019 (ICABGEH-19), Prague, Czechia, 11 – 13 September 2019, 214–216.
4. Gebreselassie, G., H. Berihulay, L. Jiang, and Y. Ma. (2020). *Review on Genomic Regions and Candidate Genes Associated with Economically Important Production and Reproduction Traits in Sheep (Ovies aries)*. Animals, 10, 33.
5. Gutiérrez-Gil, B., J.J. Arranz, R. Pong-Wong, E. García-Gámez, J. Kijas, and P. Wiener. (2014). *Application of Selection Mapping to Identify Genomic Regions Associated with Dairy Production in Sheep*. PLoS ONE 9: e94623.
6. Ibrahim, A.H.M., N. Tzanidakis, S. Sotiraki, H. Zhou, and J.G.H. Hickford. (2019). *Identification of the association between FABP4 gene polymorphisms and milk production traits in Sfakia sheep*. Arch. Anim. Breed., 62, 413–422.
7. Higgins, C.F. (1992). *ABC transporters: from microorganisms to man*. Annu Rev Cell Biol, 8, 67–113.
8. Hofmannová, M., J. Rychtářová, Z. Sztankóová, M. Milerski, L. Vostrý and A. Svitáková. (2018). *Association between polymorphism of ABCG2 gene and somatic cell count in Czech dairy sheep breeds*. Med Weter, 74(8):489–492.
9. Öner, Y., A. Orman, H. Üstüner and A. Yilmaz. (2014). *Investigation of Polymorphisms on ABCG2, AA-NAT and FABP3 Genes in the Kıvırcık Sheep Reared in Three Different Provinces of Turkey*. Kafkas Univ. Vet. Fak. Derg., 20, 649–654.
10. Mishra, C. (2014). *Genetic basis of prolificacy in sheep*. International Journal of Livestock Research, 4, 46–57.
11. Xu, S.S., and M.H. Li. (2017). *Recent advances in understanding genetic variants associated with economically important traits in sheep (Ovis aries) revealed by high-throughput screening technologies*. Front. Agric. Sci. Eng., 4, 279–288.
12. Silanikove, N., U. Merin, and G. Leitner. (2014). *On effects of subclinical mastitis and stage of lactation on milk quality in goats*. Small Rumin Res, 122, 76–82.
13. Tohidi, R., G. Elyasi, A. Javanmard, J. Shoja, R. Rezaei, and O. Pirahary. (2013). *Molecular Analysis of Ovine Calpastatin Gene in Six Iranian Sheep Breeds Using PCR-RFLP*. J. Anim. Prod. Adv, 3, 271–277.