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ACAA2 and *FASN* polymorphisms affect the fatty acid profile of Chios sheep milk

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Research Article

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Abstract

The objective of the research reported in this research communication was the identification and association of single nucleotide polymorphisms (SNP) in the ovine *DGAT1*, *FASN*, *SCD1* and *ACAA2* genes with milk fat percentage and fatty acid (FA) content. Three consecutive monthly milk samplings were obtained from a total of 429 purebred Chios ewes during mid-lactation. Genotypic data were jointly analyzed with 1184 fat content and 37 718 FA percentage records using mixed models. The 3′ untranslated region (UTR) of the *DGAT1* gene and the 5′ and 3′UTRs of the *SCD1* gene appeared to be monomorphic. The *FASN* g.14777C>T SNP on exon 31 was associated with C13:0 and the *ACAA2* g.2982T>C SNP on the 3′UTR was associated with C9:0, C11:0, C12:1 *cis*-9, C13:0 and the ω6/ω3 index, while fat percentage was not affected by the identified SNPs. The results could be useful for breeding programs aiming to improve the quality and nutritional value of ovine milk.

Milk fat and fatty acid (FA) profile affect milk and cheese quality as well as organoleptic characteristics, while specific FAs have potential health benefits. Based on studies performed mainly in cattle, some candidate genes putatively affecting the milk fat and FA content could include the acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1), the fatty acid synthase (*FASN*) and the steroyl-CoA desaturase 1 (*SCD1*) genes. Although the exon harboring *DGAT1* K232A single nucleotide polymorphism (SNP), well established to affect milk fat and FA content in dairy cattle (Schennink *et al.*, 2008), was monomorphic in some sheep breeds (Scatà *et al.*, 2009; Miltiadou *et al.*, 2010), two SNPs outside the coding region of the ovine *DGAT1* gene were associated with milk fat content (Scatà *et al.*, 2009) and a SNP in exon 17 affected milk FA traits (Dervishi *et al.*, 2015). SNPs within the *FASN* gene, catalyzing the *de novo* synthesis of small to medium chain FAs, have been associated with the bovine and ovine milk FA content (Crisà *et al.*, 2010), while the *SCD1* gene affecting the milk FA profile in dairy cows, is involved in mammary FA desaturation (Schennink *et al.*, 2008). Additionally, the acetyl-CoA acyltransferase 2 (*ACAA2*) gene, encoding an enzyme catalyzing the last step in FA β-oxidation, is located in an area where quantitative trait loci (QTL) for milk, fat and protein yields were mapped in sheep (Gutiérrez-Gil *et al.*, 2009). A SNP detected in the 3′ untranslated region (UTR) of the *ACAA2* gene was associated with milk yield, protein content and fat yield in dairy Chios sheep and exhibited differential allelic expression (Orford *et al.*, 2012; Miltiadou et al., 2017*a*).

Due to the fundamental functional role of the above genes in lipid metabolism and/or their location in chromosomal regions associated with milk content, polymorphisms within these genes may partially explain the variation of fat content and/or FA composition in sheep milk. Therefore, our objectives were to (i) identify polymorphisms in the *DGAT1*, *FASN*, *SCD1* and *ACAA2* genes and (ii) examine their association with milk fat percentage and FA profile in Chios sheep breed, a highly productive and well adapted breed of the Mediterranean region.

Material and methods

Animals, phenotypic data and sampling

Three monthly consecutive samplings from 429 purebred Chios dairy ewes in mid-lactation from four farms were obtained by machine milking twice daily and samples were kept as described in Symeou *et al.* (2019). Standard records for the date of lambing, lactation number, ewe age at lambing and date of sampling were also collected.

Table 1. Single Nucleotide Polymorphism (SNP) allelic effects in the *ACAA2* and *FASN* gene loci and corresponding percentage of total phenotypic variance explained

Trait	$a^a \pm$ SE	*P*-value Pa[b]	$d^c \pm$ SE	*P*-value Pd[d]	$\%V_p$ due to SNP[e]
	ACAA2				
C9:0	0.008 ± 0.023	0.723	−0.059 ± 0.028	0.032	0.01
C11:0	0.010 ± 0.025	0.698	−0.068 ± 0.029	0.020	0.01
C12:1 *cis*-9	0.020 ± 0.028	0.479	−0.096 ± 0.033	0.004	0.07
C13:0	0.020 ± 0.020	0.318	−0.050 ± 0.024	0.038	0.13
ω6/ω3	−0.037 ± 0.018	0.038	0.015 ± 0.021	0.469	0.76
	FASN				
C13:0	0.010 ± 0.024	0.694	−0.058 ± 0.028	0.042	0.32

[a]a = additive genetic effect; negative additive effect ($a > 0$) indicated T allele decreased the trait.
[b]*P*-value for assessing the additive effect on the trait.
[c]d = dominant genetic effect.
[d]*P*-value for assessing the dominant effect on the trait.
[e]V_p = percentage of phenotypic variance; based on the allele frequencies observed in sample (*ACAA2:* $P = 0.47$ for C and $q = 0.53$ for T; *FASN:* $P = 0.67$ for C and $q = 0.33$ for T).

DNA extraction, SNP identification and genotyping

Blood samples were collected for DNA extraction, using the Genomic DNA Blood kit (Macherey-Nagel, Duren, Germany). A cost effective direct DNA sequencing protocol (Miltiadou *et al.*, 2017*b*) was performed for SNP identification and genotyping. Thirty randomly selected animals were used to sequence the exon 10 of the *ACAA2* gene, the 3′UTR of the *DGAT1* gene, exon 31 of the *FASN* gene and the 5′ and 3′UTRs of the *SCD1* gene (online Supplementary Table S1).

Milk analysis

Milk samples were kept at 4°C for fat content determination through thermo-optical procedures (LactoStar 3510, Funke Gerber, Berlin, Germany), whereas a subsample was kept at −20°C for FA analyses as described in Tzamaloukas *et al.* (2015). At the end of the process, 1184 fat content and 37 718 FA percentage records were available for statistical analysis.

Statistical analysis

The impact of each identified genotype in each locus on the studied traits was determined using a mixed linear model presented in online Supplementary Material.

Results and discussion

The descriptive statistics obtained from Chios sheep milk regarding the mean (±SD) of fat percentage, individual FAs, FA groups and indices are presented in online Supplementary Table S2. Mean fat percentage was 5.3 (±0.9), consistent with the fat content previously recorded for the Chios breed in Cyprus (Orford *et al.*, 2012; Tzamaloukas *et al.*, 2015; Miltiadou *et al.*, 2017*a*). The milk fat fraction expressed in wt/wt included approximately 73% SFA, 26.3% UFA from which 22.4% were MUFA and 3.9% were PUFA. The highest percentage of MUFA and PUFA was oleic acid and linoleic acid, respectively, followed by CLA and linolenic acid PUFA. Similar results were previously obtained for most of the FAs from different ovine breeds (De La Fuente *et al.*, 2009) and for Chios sheep (Tzamaloukas *et al.*, 2015).

In the present work, informative polymorphisms were only observed for the *ACAA2* and *FASN* genes (Tables 1 and 2). Allelic frequencies in the *ACAA2* SNP locus were 0.47 and 0.53 for the C and T alleles, respectively, similarly to Orford *et al.* (2012) and Miltiadou *et al.* (2017*a*), while genotypic frequencies were found to deviate from Hardy-Weinberg equilibrium (online Supplementary Table S3), consistently with our latest study where the frequency of the genotypes carrying the T allele was higher probably due to directional animal selection for increased milk yield (Miltiadou *et al.*, 2017*a*). In the *FASN* SNP locus, the respective frequencies for the C and T alleles were 0.67 and 0.33 (online Supplementary Table S3), whereas the C allele frequency reported for the Altamurana and Gentile di Puglia breeds was 0.93 and 1 in Sarda breed (Crisà *et al.*, 2010), suggesting important inter-breed differences.

In this study, the UTRs of *DGAT1* and *SCD1* genes were examined for polymorphisms, since the coding regions of those genes were either monomorphic or the polymorphism had very low frequency for the minor allele in other sheep breeds studied (García-Fernández *et al.*, 2009; Scatà *et al.*, 2009; Dervishi *et al.*, 2015). However, no polymorphisms were found, which could be possibly attributed to a founder effect due to the low number of animals initially used to establish the Chios sheep population in Cyprus (Miltiadou *et al.*, 2017*a*).

In the current study, the *ACAA2* g.2982T>C SNP exhibited negative dominance effects on C9:0, C11:0, C12:1 *cis*-9 and C13:0, while a negative additive effect for the FA index ω6/ω3 was also found, explaining 0.76% of the total phenotypic variance for that index (Table 1). Therefore, the SNP previously associated with milk yield (Orford *et al.*, 2012; Miltiadou *et al.*, 2017*a*), protein content and fat yield in Chios sheep (Miltiadou *et al.*, 2017*a*) is also correlated with ω6/ω3 content, an index associated with human health benefits. Similarly to Miltiadou *et al.* (2017*a*), fat percentage was not affected by the identified *ACAA2* polymorphism.

The association detected between the *ACAA2* gene and the FA contents (odd chain FA and ω6/ω3 ratio) could be attributed to either a functional role directly affecting the studied traits or linkage and/or linkage disequilibrium with the causal locus or loci. Long chain FA reach the udder through blood circulation. Similarly, the ω6/ω3 ratio depends on the availability of ω6 and ω3 from diet, while C18:2 ω6 and C18:3 ω3 FA are metabolized

Table 2. Genotypic means for milk fatty acids (FA) and milk FA indices associated with the g.2982T/C SNP in the *ACAA2* gene locus and the g.14777C/T SNP in the *FASN* gene locus

Trait	Genotypic means ± SE[1]											
	g.2982T/C (*ACAA2*)						g.14777C/T (*FASN*)					
	CC[2]	*n*	CT[2]	*n*	TT[2]	*n*	CC[2]	*n*	CT[2]	*n*	TT[2]	*n*
C9:0	0.113 ± 0.004	191	0.109 ± 0.002	526	0.115 ± 0.004	238						
C11:0	0.136 ± 0.005	192	0.128 ± 0.003	530	0.137 ± 0.005	229						
C12:1 *cis*-9	0.152 ± 0.005	190	0.143 ± 0.003	540	0.151 ± 0.005	239						
C13:0	0.089 ± 0.003	193	0.086[b] ± 0.002	539	0.094[a] ± 0.003	245	0.088 ± 0.002	405	0.083 ± 0.002	423	0.090 ± 0.005	91
ω6/ω3	13.67 ± 0.29	178	13.58 ± 0.18	508	12.95 ± 0.29	221						

[a,b]Means within a row with 2 different superscripts differ as follows: [a,b]$P < 0.05$.
[1]Predicted from the mixed model association analyses, after adjusting for all significant fixed and random effects.
[2]Significance of pairwise genotype contrast assessed using a 2-sample *t*-test.

to longer chain ω6 and ω3 FA (Palmquist, 2009). Therefore, acetyl-CoA produced in mitochondria by the *ACAA2* enzyme, could be used in the formation of malonyl-CoA, utilized for the FA chain elongation (Eaton *et al.*, 1996). Therefore, the *ACAA2* g.2982T>C SNP may be indirectly associated with the ω6/ω3 ratio by producing the substrates for elongation of the C18:2 ω6 and C18:3 ω3 precursors. On the same basis, even-chain and odd-chain FA are elongated with the aid of acetyl-CoA. Furthermore, β oxidation provides energy for such anabolic reactions.

In the current study, we confirm the presence of the g.14777C>T SNP (Crisà *et al.*, 2010) in the *FASN* gene of Chios sheep and provide evidence of a significant association with an odd chain FA (C13:0) with a negative dominant effect (Tables 1 and 2), explaining 0.32% of the total phenotypic variance. Crisà *et al.* (2010) described that the allele T of the *FASN* SNP in exon 31 had a positive substitution effect on the medium-chain milk FA (C10:0, C10:1, C12:0 and C14:0) in Altamurana and Gentile di Puglia sheep. Odd-chain FAs are in low percentage in milk compared to the corresponding FA with even number of carbon atoms that have been previously associated with *FASN* polymorphism (Crisà *et al.*, 2010). Although the majority of odd-chain FA derive from ruminal bacteria, another small proportion is *de novo* synthesized (C5:0 to C13:0) from propionate in the mammary gland of ruminants (Vlaeminck *et al.*, 2006). Heck *et al.* (2012) also suggested that odd-chain FA are *de novo* synthesized as a much larger proportion of their variance is explained by genetic rather than herd parameters, suggesting a possible involvement of *FASN* in odd chain FA synthesis.

Studies in cows have yielded important findings for consistent and well established gene effects on the milk FA profile. This kind of knowledge would be useful for the small ruminant industry as it could possibly facilitate the improvement of milk and cheese FA composition. In the case of dairy sheep, a large number of locally adapted breeds are commonly used in different countries and, therefore, breed specific associations have been detected. In the present study, we report the association between polymorphisms within the *ACAA2* and *FASN* genes and milk fatty acid profile in ewe milk. The effect of *ACAA2* SNP on ω6/ω3 content, an index with well-established effects in human health, could be possibly incorporated in a selection scheme including other SNPs affecting the ω6/ω3 index toward improvement of milk FA quality. Further studies are needed to investigate if the association of the *ACAA2* gene with the FA profile is breed specific or could also be observed in other ovine breeds.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029919000992.

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References

Crisà A, Marchitelli C, Pariset L, Contarini G, Signorelli F, Napolitano F, Catillo G, Valentini A and Moioli B (2010) Exploring polymorphisms and effects of candidate genes on milk fat quality in dairy sheep. *Journal of Dairy Science* **93**, 3834–3845.

De La Fuente LF, Barbosa E, Carriedo JA, Gonzalo C, Arenas R, Fresno JM and San Primitivo F (2009) Factors influencing variation of fatty acid content in ovine milk. *Journal of Dairy Science* **92**, 3791–3799.

Dervishi E, Serrano M, Joy M, Sarto P, Somera A, González-Calvo L, Berzal-Herranz B, Molino F, Martinez-Royo A and Calvo JH (2015) Structural characterisation of the acyl CoA: diacylglycerol acyltransferase 1 (DGAT1) gene and association studies with milk traits in Assaf sheep breed. *Small Ruminant Research* **131**, 78–84.

Eaton S, Bartlett K and Pourfarzam M (1996) Mammalian mitochondrial beta-oxidation. *The Biochemical Journal* **320**, 345–357.

García-Fernández M, Gutiérrez-Gil B, García-Gámez E and Arranz JJ (2009) Genetic variability of the Stearoyl-CoA desaturase gene in sheep. *Molecular and Cellular Probes* **23**, 107–111.

Gutiérrez-Gil B, El-Zarei MF, Alvarez L, Bayón Y, de La Fuente LF, San Primitivo F and Arranz JJ (2009) Quantitative trait loci underlying milk production traits in sheep. *Animal Genetics* **40**, 423–434.

Heck JML, van Valenberg HJF, Bovenhuis H, Dijkstra J and van Hooijdonk TCM (2012) Characterization of milk fatty acids based on genetic and herd parameters. *Journal of Dairy Research* **79**, 390–346.

Miltiadou D, Hager-Theodorides AL, Symeou S, Constantinou C, Psifidi A, Banos G and Tzamaloukas O (2017a) Variants in the 3′ untranslated region of the ovine acetyl-coenzyme A acyltransferase 2 gene are associated with dairy traits and exhibit differential allelic expression. *Journal of Dairy Science* **100**, 6285–6297.

Miltiadou D, Orford M, Symeou S and Banos G (2017b) Short communication: identification of variation in the ovine prolactin gene of Chios sheep with a cost-effective sequence-based typing assay. *Journal of Dairy Science* **100**, 1290–1294.

Miltiadou D, Tzamaloukas O, Genethliou N, Papachristoforou C, Chatziplis D and Orford M (2010) Do established SNPs affecting bovine milk traits exist in other dairy ruminants? *Small Ruminant Research* **88**, 72–74.

Orford M, Hadjipavlou G, Tzamaloukas O, Chatziplis D, Koumas A, Mavrogenis A, Papachristoforou C and Miltiadou D (2012) A single nucleotide polymorphism in the acetyl-coenzyme A acyltransferase 2 (ACAA2) gene is associated with milk yield in Chios sheep. *Journal of Dairy Science* **95**, 3419–3427.

Palmquist DL (2009) Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. *The Professional Animal Scientist* **25**, 207–249.

Scatà MC, Napolitano F, Casu S, Carta A, De Matteis G, Signorelli F, Annicchiarico G, Catillo G and Moioli B (2009) Ovine acyl CoA: diacylglycerol acyltransferase 1- molecular characterization, polymorphisms and association with milk traits. *Animal Genetics*, **40**, 737–742.

Schennink A, Heck JML, Bovenhuis H, Visker MHPW, van Valenberg HJF and van Arendonk JAM (2008) Milk fatty acid unsaturation: genetic parameters and effects of stearoyl-CoA desaturase (SCD1) and acyl CoA: diacylglycerol acyltransferase 1 (DGAT1). *Journal of Dairy Science* **91**, 2135–2143.

Symeou S, Tsiafoulis CG, Gerothanassis IP, Miltiadou D and Tzamaloukas O (2019) Nuclear magnetic resonance screening of changes in fatty acid and cholesterol content of ovine milk induced by ensiled olive cake inclusion in Chios sheep diets. *Small Ruminant Research* **177**, 111–116.

Tzamaloukas O, Orford M, Miltiadou D and Papachristoforou C (2015) Partial suckling of lambs reduced the linoleic and conjugated linoleic acid contents of marketable milk in Chios ewes. *Journal of Dairy Research* **98**, 1739–1749.

Vlaeminck B, Fievez V, Cabrita ARJ, Fonseca AJM and Dewhurst RJ (2006) Factors affecting odd- and branched-chain fatty acids in milk: a review. *Animal Feed Science and Technology* **131**, 389–417.