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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

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ABSTRACT

The acetyl-CoA acyltransferase 2 (*ACAA2*) gene encodes an enzyme of the thiolase family that is involved in mitochondrial fatty acid elongation and degradation by catalyzing the last step of the respective β-oxidation pathway. The increased energy needs for gluconeogenesis and triglyceride synthesis during lactation are met primarily by increased fatty acid oxidation. Therefore, the *ACAA2* enzyme plays an important role in the supply of energy and carbon substrates for lactation and may thus affect milk production traits. This study investigated the association of the *ACAA2* gene with important sheep traits and the putative functional involvement of this gene in dairy traits. A single nucleotide substitution, a T to C transition located in the 3′ untranslated region of the *ACAA2* gene, was used in mixed model association analysis with milk yield, milk protein yield and percentage, milk fat yield and percentage, and litter size at birth. The single nucleotide polymorphism was significantly associated with total lactation production and milk protein percentage, with respective additive effects of 6.81 ± 2.95 kg and −0.05 ± 0.02%. Additionally, a significant dominance effect of 0.46 ± 0.21 kg was detected for milk fat yield. Homozygous TT and heterozygous CT animals exhibited higher milk yield compared with homozygous CC animals, whereas the latter exhibited increased milk protein percentage. Expression analysis from age-, lactation-, and parity-matched female sheep showed that mRNA expression of the *ACAA2* gene from TT animals was 2.8 and 11.8 fold higher in liver and mammary gland, respectively. In addition, by developing an allelic expression imbalance assay, it was estimated that the T allele was expressed at an average of 18% more compared with the C allele in the udder of randomly selected ewes. We demonstrated for the first time that the variants in the 3′ untranslated region of the ovine *ACAA2* gene are differentially expressed in homozygous ewes of each allele and exhibit allelic expression imbalance within heterozygotes in a tissue-specific manner, supporting the existence of *cis*-regulatory DNA variation in the ovine *ACAA2* gene. This is the first study reporting differential allelic imbalance expression of a candidate gene associated with milk production traits in dairy sheep.

Key words: *ACAA2* association, 3′ untranslated region *cis*-acting SNP, gene expression, dairy sheep

INTRODUCTION

Milk yield represents more than two-thirds of the total income of the dairy sheep industry (Carta et al., 2009); therefore, the improvement of milk production is the most important breeding objective. In Mediterranean countries, however, most of the ovine milk produced is used for the production of cheese commercialized as products of protected designation of origin (PDO) and other quality labels (Arranz and Gutiérrez-Gil, 2012). Thus, farmers' income is additionally determined by TS that affect cheese yield (De Rancourt et al., 2006); therefore, increased milk fat and protein content is also highly desirable from an economic perspective (Ramón et al., 2010). Although traditional breeding programs have achieved appreciable genetic gains mainly for milk yield, application of selection schemes assisted by molecular information could expedite improvement (Carta et al., 2009). Moreover, marker-assisted selection could be of special interest for dairy sheep due to the great regional diversity of breeds, funding limitations, organizational difficulties (Arranz and Gutiérrez-Gil, 2012), and small population sizes (Garciá-Gámez et al., 2012).

To date, few reports exist of genome-wide association studies and genome scans based on linkage mapping that

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detect QTL and quantitative trait mutations for ovine dairy traits (reviewed by Arranz and Gutiérrez-Gil, 2012; Garciá-Gámez et al., 2012, 2013; Gutiérrez-Gil et al., 2014). In a whole-genome QTL study performed in Churra ewes, Gutiérrez-Gil et al. (2009) detected suggestive QTL for milk, fat, and protein yields, mapped in a region of the ovine chromosome 23 harboring the acetyl-CoA acyltransferase 2 (*ACAA2)* gene. The *ACAA2* gene encodes an enzyme of the thiolase family, also known as 3-oxoacyl-CoA thiolase or mitochondrial 3-ketoacyl-CoA thiolase. The *ACAA2* enzyme catalyzes the last step in mitochondrial fatty acid β-oxidation, thus playing a central role in the supply of energy for the animal (Bartlett and Eaton, 2004). Therefore, due to the chromosomal location of the ovine *ACAA2* gene in relation to the QTL described by Gutiérrez-Gil et al., (2009) and its functional role in lipid metabolism, it was regarded as a putative functional and positional candidate gene that may affect milk yield and composition.

Genes encoding enzymes of the thiolase family have been correlated with production traits in other livestock species. Single nucleotide polymorphisms detected in the porcine *ACAA2* gene are reported to be associated with daily weight gain and loin muscle area (Li HD, 2008). An important paralog of the *ACAA2* gene, the acetyl-CoA acetyltransferase 2 (*ACAT2*) gene, has been associated with production and fertility traits (milk protein content, productive life, and conception and pregnancy rates) in Holstein cattle (Cochran et al., 2013), whereas SNP within the swine *ACAT2* gene were suggested to influence the metabolic functions of the corresponding enzyme and thus may affect growth performance (Sodhi et al., 2014).

Our previous study showed that the entire mRNA (coding and untranslated regions) of the ovine *ACAA2* gene is monomorphic in Chios sheep, one of the most productive and extensively used breeds in Greece and Cyprus (Chatziplis et al., 2012), with the exception of a SNP (HM537015:g.2982T > C) located in the 3′ untranslated region (**UTR**) of the gene (Orford et al., 2012). The SNP was significantly associated with milk yield at first lactation and across first to third lactations in Chios sheep. Animals from a closed nucleus research flock at the Agricultural Research Institute of Cyprus (Nicosia) carrying the g.2982TT or g.2982CT genotype had significantly higher milk yield than those with the g.2982CC genotype, and the g.2982T > C SNP explained 10% of the additive genetic variance for milk yield when data up to third lactation from a single flock were analyzed (Orford et al., 2012).

It is well established that UTR contain motifs involved in posttranscriptional regulation of gene expression (Xie et al., 2005) that may lead to differential expression of alleles associated with phenotypic diversity of production traits (Clop et al., 2006; Khatib et al., 2007; Sugimoto et al., 2015). Studies in humans (Yan et al., 2002; Bray et al., 2003), mice (Cowles et al., 2002), cattle (Khatib et al., 2007; Olbromski et al., 2013), and pig (Muráni et al., 2009) have shown that alleles of nonimprinted genes are not expressed equally at the mRNA level in heterozygous animals, a phenomenon called allelic expression imbalance (**AEI**). Allelic expression imbalance is the outcome of the presence of at least 1 *cis* regulatory element in the regulatory sequences of a gene (Campbell et al., 2008); therefore, AEI is one of the possible mechanisms underlying the effect of causative genetic variations that are not located on the translated region of a gene.

The objective of the current study was to provide novel insights into the association of the ovine *ACAA2* gene with important sheep traits. First we performed an association analysis of the previously identified g.2982T > C SNP with milk yield, fat and protein contents, fat and protein yields, and litter size in a population of Chios sheep from all available farms keeping production records in Cyprus. Upon confirmation of the association of the SNP with total milk production and additional detection of association with milk protein percentage, we tested the hypothesis that the *ACAA2* is a putative functional gene affecting dairy traits by comparing the expression of the gene in different genotypes and by developing an assay to test the presence of AEI.

MATERIALS AND METHODS

Animals and Phenotypic Data

Data were collected from 742 purebred Chios ewes from 5 farms. The farms included 1 governmental farm (Orites, Cyprus) and the only 4 commercial farms in Cyprus keeping phenotypic records of purebred Chios sheep according to the regulations of the International Committee for Animal Recording (ICAR, 2014).

For all animals, individual records included month of lambing, year of lambing, lactation number, and age of lambing. Phenotypic data were obtained for lambing years between 2009 and 2016 and included 1,514 individual records of 742 milking ewes; this data included total lactation milk yield, 1,242 observations for litter size at birth, 1,203 measurements of milk fat content and yield, and 615 measurements of milk protein content and yield, respectively. Total lactation yield was calculated for each animal with the Fleischmann method with monthly tests on actual yields (sum of a.m. and p.m. records, ICAR 2014). Milk samples were obtained for fat and protein analysis by combined thermo-optical procedures (LactoStar 3510, Funke Gerber, Berlin,

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