Association Between *Acetyl-CoA Carboxylase α (ACACA)* SNPs and Milk Fatty Acid Profile in Spanish Churra Sheep

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Introduction

Sheep milk is mainly processed into high quality cheeses, which can be considered an unhealthy product by some consumers due to its high fat content. On average, the sheep milk fat content is composed of more than 60% of saturated fatty acids (SFA), 28% of monounsaturated fatty acids (MUFA) and 6% of polyunsaturated fatty acids (PUFA) (Cabiddu et al., 2005). However, sheep milk fat also contains several components that may provide benefits to human health, such as MUFAs and Conjugated linoleic acid (CLA). Many efforts are being addressed to increase the content of “healthy” fatty acids (FA) in sheep milk by means of nutrition (Chilliard et al., 2000). Animal breeding has been considered as an alternative to modify FA profile in sheep milk. However, in previous studies (Carta et al., 2008; Sánchez et al., 2010), it has been shown that low genetic variation is involved in the control of these traits. In spite of this, the usage of molecular tools could help deciphering the genetic basis of fat milk FA composition. One of the most important enzymes involved in the synthesis of milk FA, is Acetyl-CoA carboxylase alpha (ACACA), which is the rate-limiting enzyme in the biosynthesis of palmitic acid (C16:0) and long-chain FA. The ACACA enzyme is expressed ubiquitously but highest levels are found in lipogenic tissues like liver, adipose tissue and mammary gland during lactation. ACACA catalyzes the ATP-dependent carboxylation of acetyl-CoA to form malonil-CoA, which is the substrate for the synthesis of palmitic acid and very long chain FA (acyl-CoA >C22:0) by the fatty acid synthase (FAS) enzyme (Smith et al., 2003; Leonard et al., 2004). In sheep, this enzyme is codified by a single gene located on chromosome 11 (OAR11), which has 54 exons and encodes a protein of 2346 amino acids (Barber and Travers, 1995). In a previous analysis involving 24 non-related Spanish Churra individuals we have identified a total of 22 single nucleotide polymorphisms (SNPs) across the 6.6 Kb of the ovine ACACA cDNA sequence analysed (García-Fernández et al., in press). The objective of this study is to assess any association between the identified SNPs and 22 measurements related to milk FA composition and three milk production traits (milk protein percentage, milk fat percentage and milk yield).

Material and methods

Animals and Phenotypic Data

A total of 799 ewes belonging to 16 flocks owned by Churra Association Breeders were genotyped for association analysis. All the animals belonged to 15 half-sib families.

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generated by AI and ranging from 29 to 131 ewes per family. All these females had milk yield, protein percentage and fat percentage records obtained within the official Churra selection scheme (Gutiérrez-Gil et al., 2009). The milk fatty acid composition was determined as explained by De la Fuente et al. (2009). Briefly, after extraction of total fat milk, the fatty acid content (grams per 100 g of total FA) was determined by gaseous chromatography, after methylation of free fatty acids with NaOCH₃. For this measurement, a Hewlett Packard 6890 Series GC System chromatographer was used. In this work, 22 FA measurements were included: 15 individual FA, 3 groups of FA based on the saturation level (SFA, MUFA and PUFA); and 4 FA indexes, α6/α3, C14:1/C14:0, C16:1/C16:0 and CLA/VA ratio.

**Association Analysis**

The effect of the 22 genotyped ACACA gene SNPs on fat FA composition and milk production traits was estimated individually assuming the following univariate sire repeatability model:

\[ y_{ijklmn} = HTD_i + DIM_j + AGE_k + s_m + p_l + \beta \times SNP + e_{ijklmn} \]

where the considered fixed effects were Herd-Test-Day (HTD, 157 levels), the effect of days in milk (DIM, 7 levels) and the age of the ewe at parturition (AGE, 6 levels); \( p_l \) is the permanent environmental effect for animal \( l \), \( s_m \) refers to the ram \( m \) siring ewe \( l \), \( \beta \) is the allele substitution effect of the studied locus, being \( SNP \) a covariate reflecting the number of copies of a particular allele that the animal \( l \) is carrying at the studied locus (e.g., 1, GG; 2, AG; or 3, AA). The needed variance components to solve this mixed model were estimated using the program REMLf90 (Misztal, 1998). Once they were known, a fixed equivalent model was implemented to test (using F test) for the significance of the allele substitution effect. R package (R Development Core Team, 2008) was used both for solving the equivalent fixed model and for conducting the F test. Finally, to take into account the fact that multiple tests were conducted (a total of 484, once for each combination of trait and SNP locus) a Bonferroni correction, considering the number of independent traits and SNPs, was performed.

**Results and discussion**

The association analyses performed in the present study revealed 15 significant associations at the 5% nominal level, which are summarized in Table 1. The most important association was found between SNP c.1441C>T and the alpha linolenic acid (C18:3 cis-9,12,15) content (p-value = 0.0048). This SNP was also associated with the linoleic acid (C18:2 cis-9,12) content. The second most important association involved the SNP c.4579G>A, which is located at exon 35, and the lauric acid (C12:0) content (p-value = 0.0145). This SNP also influences on two other FAs related traits (C10:0, C18:3 cis-9,12,15) and the milk fat percentage. Another SNP influencing several of the studied traits was SNP c.6481C>T, in particular it influences the myristic acid (C14:0) content, the saturated fatty acid (SFA) content and the milk protein percentage. The magnitude of the allelic substitution effects estimated for the described associations ranged from 0.0087 (for the milk protein percentage and SNP c.6481C>T association) to 0.206 (for the SFA and SNP c.6481C>T association).
Intriguingly, none of the identified SNPs was associated to the palmitic acid (C16:0) content as would be expected on the basis of the biological function of the ACACA enzyme.

Table 1: List of significant associations (nominal P-values) between ACACA SNPs and milk traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Common name</th>
<th>SNP*</th>
<th>p-value</th>
<th>Allelic substitution effect ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0</td>
<td>Capric acid</td>
<td>c.4579G&gt;A</td>
<td>0.0434</td>
<td>(-0.074 ± 0.0359)</td>
</tr>
<tr>
<td>C12:0</td>
<td>Lauric acid</td>
<td>c.4579G&gt;A</td>
<td>0.0145</td>
<td>(0.074 ± 0.0297)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.3795C&gt;T</td>
<td>0.0367</td>
<td>(-0.157 ± 0.0742)</td>
</tr>
<tr>
<td>C14:0</td>
<td>Myristic acid</td>
<td>c.6481C&gt;T</td>
<td>0.0171</td>
<td>(0.116 ± 0.0477)</td>
</tr>
<tr>
<td>C18:1 cis-9</td>
<td>Oleic acid</td>
<td>c.2347C&gt;T</td>
<td>0.0272</td>
<td>(-0.100 ± 0.0456)</td>
</tr>
<tr>
<td>C18:2 cis-9,12</td>
<td>Linoleic acid</td>
<td>c.1441C&gt;T</td>
<td>0.0314</td>
<td>(0.058 ± 0.0262)</td>
</tr>
<tr>
<td>C18:3 cis-9,12,15</td>
<td>α-linoleic acid</td>
<td>c.1441C&gt;T</td>
<td>0.0048</td>
<td>(0.047 ± 0.0171)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.4579G&gt;A</td>
<td>0.0307</td>
<td>(0.162 ± 0.0754)</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated fatty acids</td>
<td>c.6481C&gt;T</td>
<td>0.0488</td>
<td>(0.206 ± 0.1054)</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated FA</td>
<td>c.3823C&gt;T</td>
<td>0.0200</td>
<td>(0.151 ± 0.0664)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.6412A&gt;G</td>
<td>0.0346</td>
<td>(0.065 ± 0.0317)</td>
</tr>
<tr>
<td>Protein percentage</td>
<td>Prot. %</td>
<td>c.1834T&gt;C</td>
<td>0.0244</td>
<td>(0.057 ± 0.0251)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.6481C&gt;T</td>
<td>0.0232</td>
<td>(0.009 ± 0.0376)</td>
</tr>
<tr>
<td>Fat percentage</td>
<td>Fat %</td>
<td>c.4579G&gt;A</td>
<td>0.0475</td>
<td>(0.119 ± 0.0587)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.7021C&gt;T</td>
<td>0.0427</td>
<td>(0.098 ± 0.0474)</td>
</tr>
</tbody>
</table>

*(positions according GenBank Acc. No. NM001009256.1)

When a Bonferroni correction was performed to take into account that multiple traits and SNPs were analyzed, none of the associations remained significant. However, note that this procedure is too conservative and its rate of false negative is too high.

To our knowledge there are not reports in the literature of significant associations between this gene and milk fatty acid composition in ruminants. In goats, suggestive relationships between SNPs of the ACACA gene and milk traits have been reported by Badaoui et al. (2007) and Federica et al. (2009). However, several studies have identified relationships between the ACACA gene and meat fatty acid composition. Zhang et al. (2009) identified several SNPs in the promoter I of the bovine ACACA gene, some of which showed significant associations with the content of several saturated and polyunsaturated fatty acids. In pig, Muñoz et al. (2007) and Gallardo et al. (2009) found significant associations between two linked SNPs in the ACACA gene and the PUFA and ω-6 contents and the PUFA/MUFA ratio.

Regarding the Churra population the weak associations observed in this study are in line with the low additive genetic variance shown to be involved in the control of these traits by
Sánchez et al. (2010), and also with the weak associations observed between FA traits and others genes codifying for essential enzymes in the biosynthesis of certain FAs (Stearoyl-CoA desaturase, García-Fernández et al., 2009).

Conclusion

ACACA gene polymorphisms do not show a strong association neither with fatty acid profile of Churra sheep milk or with milk production traits. Suggesting that polymorphic information regarding this gene would have a limit value when used to alter the FA profile within a selection scheme in this breed.

References