Small Ruminant Research 121 (2014) 244–247

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Short communication

An association analysis between the variability of the caprine *CD36* and *CD36-like* genes and dairy traits

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ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 30 June 2014
Accepted 8 July 2014
Available online 17 July 2014

Keywords:
Goat CD36
Milk fat composition
Association analysis
Polymorphism

ABSTRACT

The CD36 molecule plays a key role in the uptake of long-chain fatty acids. In a previous study, we demonstrated that the *CD36* gene is duplicated in goats. Moreover, both copies (*CD36* and *CD36-like*) display highly divergent mRNA expression profiles. Herewith, we have analyzed whether four polymorphisms mapping to *CD36* (c.394A > G, c.*141C > T and c.*427T > A) and *CD36-like* (c.390A > C) genes are associated with milk yield and composition. Murciano-Granadina goats with records for dairy traits ($N = 309$, 1005 registers) and milk fatty acid composition phenotypes ($N = 176$, 490 registers) were genotyped for these four markers and association analyses were carried out. We found a highly significant association between c.*427T > A *CD36* polymorphism and milk palmitoleic content ($P < 0.0001$). Besides, the c.*141C > T *CD36* SNP also showed a suggestive association ($P = 0.04$) with palmitoleic. These findings are consistent with previous studies showing that *CD36* inactivation in adipocytes and neurons involves a decrease in palmitoleic content, thus suggesting a relevant role of this molecule in the specific uptake of this fatty acid. The remaining associations found (*CD36* with polyunsaturated fatty acids and *CD36-like* with oleic and several saturated fatty acids) were significant at the nominal level but not after Bonferroni correction. These results, combined with previously reported expression data, reinforce the interest of investigating the lipid binding properties of CD36 and CD36-like through functional approaches.

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1. Introduction

The thrombospondin receptor (CD36) has a broad repertoire of metabolic and immunological functions related with the binding of diverse ligands such as ionized long-chain fatty acids (FA), collagen, anionic phospholipids and oxidized low-density lipoproteins (Silverstein and

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http://dx.doi.org/10.1016/j.smallrumres.2014.07.008
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Febbraio, 2009; Buttet et al., 2014). This glycoprotein harbours two transmembrane domains plus an extracellular functional domain containing one thrombospondin-binding site and a large hydrophobic pocket that can attach to saturated and unsaturated FA (Martin et al., 2011). CD36 has been also implicated in the chemosensory perception of dietary lipids by the gustatory papillae, oral fat perception, hepatic lipoprotein production, activation of muscle FA β-oxidation and chylomicron synthesis (Pepino et al., 2014). Human CD36 variability has been associated with free FA plasma levels (Ma et al., 2004), triglyceride and high density lipoprotein concentrations (Love-Gregory et al., 2008) and body weight (Bokor et al., 2009).

In cattle, mammary gland *CD36* mRNA expression increases very significantly during lactation, confirming the essential role of this receptor in the uptake of FA for the synthesis of milk fat (Bionaz and Loor, 2008). Recently, Zidi et al. (2013) demonstrated that the *CD36* locus is duplicated in cattle and goats and identified two gene copies named as *CD36* and *CD36-like*. In goats, these two genes encode thrombospondin receptors with 84% amino acid identity and highly divergent mRNA expression profiles, a feature that suggests that they may fulfil different biological functions (Zidi et al., 2013). Moreover, Zidi et al. (2013) reported the existence of variation at these two loci *i.e.* c.394A > G (p.N132D), c.*141C > T and c.*427T > A single nucleotide polymorphisms (SNPs) at *CD36* and c.390A > C SNP at *CD36-like*. The main goal of the current work was to investigate whether these four polymorphisms are associated with milk yield and composition in Murciano-Granadina goats.

2. Materials and methods

2.1. Measurement of milk traits in Murciano-Granadina goats

Milk traits were registered in two goat populations: Population 1 consisted of 133 goats belonging to three disconnected herds (Badaoui et al., 2007), with each goat having around 3–4 measurements (Badaoui et al., 2007). All the procedures used to determine milk phenotypes in Population 1 have been described by Badaoui et al. (2007). The second population (Population 2) was constituted by one herd of 176 goats (around 2–3 registers per goat were available). Phenotype recording was initiated three months post-partum. A MilkoScan FT 6000 instrument was used to measure protein, fat, lactose and dry matter contents.

The composition of milk fat was estimated only in Population 2. Separation and quantification of FA methyl esters were carried out with a gas chromatograph Agilent 6890 N Network GS System (Agilent, Santa Clara, CA), equipped with a flame ionization detector and fitted with a HP-88 capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness). Nonanoic acid methyl ester (C9:0 ME, 4 mg/ml) was employed as an internal standard. Extraction and direct methylation were performed in a single step method following the procedures reported by Sukhija and Palmquist (1998). Individual FA were identified by comparing their retention times with those of an authenticated standard FA mix Supelco 37 (Sigma Chemical Co. Ltd., Poole, UK). Identification of the CLA isomers 9cis-11trans, 11cis-13trans, 10trans-12cis and 10cis-12cis CLA was achieved by comparing retention times with those of another authenticated standard mix (Sigma Chemical Co. Ltd., Poole, UK). Fatty acid content was expressed as the percentage of total FA identified. Performed and *de novo* FA were calculated as indicated by Moate et al. (2007)

2.2. Genotyping of the goat CD36 and CD36-like genes

Genomic DNA was purified from blood samples following Caravaca et al. (2009). The amplification profiles of *CD36* and *CD36-like* regions containing SNPs consisted of 35 cycles of 94 °C for 1 min, annealing temperature (Supplementary Table 1) for 1 min and 72 °C for 2 min.

Table 1
Associations between goat *CD36* genotypes (number of individuals and records are shown in parentheses) and milk fatty acid traits (Lsmeans ± SE).

Milk phenotypes	c.394A > G			c.*141C > T				c.*427T > A		
	AA (32, 83)	AG (140, 396)	*P*	CC (80, 227)	CT (85, 232)	TT (11, 31)	*P*	AT (26, 70)	TT (144, 402)	*P*
C11:0	0.12 ± 0.01	0.12 ± 0.01	0.65	0.12 ± 0.01[b]	0.12 ± 0.01[b]	0.19 ± 0.02[a]	**0.01**	0.13 ± 0.02	0.12 ± 0.01	0.67
C12:0	5.75 ± 0.21	5.87 ± 0.17	0.46	5.94 ± 0.18[a]	5.78 ± 0.18[a]	5.22 ± 0.30[b]	**0.03**	6.19 ± 0.23[a]	5.77 ± 0.17[b]	**0.02**
C14:0	10.13 ± 0.21	10.32 ± 0.17	0.27	10.32 ± 0.18	10.29 ± 0.18	9.95 ± 0.30	0.39	10.76 ± 0.22[a]	10.22 ± 0.16[b]	**0.02**
C14:1	0.15 ± 0.009	0.14 ± 0.007	0.44	0.15 ± 0.008	0.14 ± 0.007	0.15 ± 0.01	0.09	0.16 ± 0.01[a]	0.14 ± 0.007[b]	**0.01**
C16:1	0.91 ± 0.03	0.92 ± 0.02	0.87	0.95 ± 0.03[a]	0.89 ± 0.02[b]	0.93 ± 0.05[ab]	**0.04**	1.03 ± 0.03[a]	0.90 ± 0.02[b]	**<0.0001**
C18:0	10.76 ± 0.34	10.40 ± 0.28	0.18	10.28 ± 0.29	10.60 ± 0.28	10.52 ± 0.48	0.31	9.91 ± 0.36[b]	10.54 ± 0.27[a]	**0.03**
C18:2n6c	2.30 ± 0.07[b]	2.44 ± 0.06[a]	**0.02**	2.40 ± 0.06	2.42 ± 0.06	2.34 ± 0.11	0.70	2.35 ± 0.08	2.42 ± 0.06	0.29
C18:3n3a	0.19 ± 0.009[b]	0.21 ± 0.008[a]	**0.04**	0.21 ± 0.008	0.20 ± 0.008	0.20 ± 0.01	0.77	0.21 ± 0.01	0.20 ± 0.007	0.15
C18:3n6 g	0.06 ± 0.008	0.06 ± 0.007	0.85	0.06 ± 0.007	0.06 ± 0.007	0.07 ± 0.01	0.12	0.05 ± 0.009[b]	0.06 ± 0.006[a]	**0.04**
9-cis11-trans CLA	0.40 ± 0.03	0.40 ± 0.03	0.94	0.38 ± 0.03	0.39 ± 0.03	0.50 ± 0.05	0.59	0.33 ± 0.04[b]	0.40 ± 0.03[a]	**0.04**
PUFA	3.88 ± 0.11[b]	4.08 ± 0.09[a]	**0.04**	4.01 ± 0.10	4.02 ± 0.10	4.13 ± 0.17	0.72	3.91 ± 0.13	4.04 ± 0.09	0.19
Omega 6	2.81 ± 0.09[b]	2.99 ± 0.08[a]	**0.02**	2.96 ± 0.08	2.94 ± 0.08	2.96 ± 0.14	0.93	2.89 ± 0.10	2.95 ± 0.08	0.43
De novo FA	33.53 ± 0.76	34.57 ± 0.62	0.28	34.68 ± 0.66	34.29 ± 0.64	32.74 ± 1.09	0.13	35.56 ± 0.82[a]	34.21 ± 0.60[b]	**0.03**
Performed FA	33.85 ± 0.73	33.38 ± 0.60	0.42	33.27 ± 0.63	33.73 ± 0.62	34.01 ± 1.05	0.53	32.37 ± 0.79[b]	33.73 ± 0.58[a]	**0.03**

Means within rows with different superscripts [a,b] show either significant ($P < 0.0013$) or suggestive ($P < 0.05$) differences.

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