

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

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5 **SUPPLEMENTARY FILE**

6 **Symeou5747**

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8 ***Statistical analysis***

9 The following mixed linear model was used for the determination of the impact of each genotype in the
10 g.2982T>C ACAA2 locus and in the g.14777C>T FASN locus; Each trait was analyzed separately:

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$$Y_{ijklmn} = \mu + F_i + YS_j + L_k + b_{1age} + G_l + A_m + e_{ijklmn} \quad [1]$$

12 Where:

13 Y refers to the phenotypes of fat percentage and 43 individual milk fatty acids, 9 fatty acid groups
14 (saturated FA (SFA), unsaturated FA (UFA), MUFA, PUFA, short chain FA (SCFA), mammary origin
15 FA, medium chain FA (MCFA), long chain FA (LCFA), Odd chain FA) and 3 constructed indices
16 ($\omega 6/\omega 3$, atherogenic index (AI), elongation index (EI));

17 μ = overall population mean for the trait;

18 F = fixed effect of flock *i* (1–4);

19 YS = fixed effect of the year (2012-2013) by season (1–2) of lambing interaction *j*;

20 L = fixed effect of lactation number *k* (1–7);

21 b_1 = linear regression on age at lambing (age);

22 G = fixed effect of genotype *l* (1–3; CC, CT, TT);

23 A = random effect of animal m ; e = random residual effect.

24 The predicted values for all traits and their respective standard errors were derived for each SNP
25 genotype in each locus and reflected the marginal genotypic effect on the trait adjusted for all other
26 effects fitted in the model. Equations [2] and [3] were applied on the predicted phenotypic values for the
27 estimation of the additive (a) and dominance (d) SNP effects on traits. The proportion of phenotypic
28 variance (V_p) for each trait accounted for by the SNP locus was estimated with the equation [4].

29 $a = (TT - CC)/2,$ [2]

30 $d = CT - [(TT + CC)/2],$ [3]

31 $V_p \% \text{ due to SNP} = 100 \times \{2pq[a + d(q - p)]^2\}/V_p$ [4]

32 where TT, CC, and CT are the predicted trait values for each genotype class; p and q are the respective
33 allele frequencies at the SNP locus. The statistical model [1] was used to estimate the variance
34 components after excluding the genotype effect. For all statistical analyses the ASReml3 software was
35 used. Pairwise t-test between the genotype classes (TT, CT and CC) was performed on the corresponding
36 predicted trait values.

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45 **Supplementary Table S1.** Primers and reaction conditions for PCR used to amplify the area of interest of the *ACAA2*, *DGAT1*, *FASN* and *SCD*
 46 genes

Primer	Sequence 5'→3'	SNP ¹	OAR ²	Annealing T (°C) ³	MgCl (mM)	Amplicon size (bp)	Covered region ⁴	GenBank accession no.
For SNP identification								
ACAA2_3'UTR_F1 ACAA2_3'UTR_R1	TTGATTATCTAATTCCGTACATTCTTT TTTAATGCCACTGAAGTGTATGC	g.2982T>C	23	55	2.5	598	exon 10 - 3' UTR	HM537015.2
DGAT1_3'UTR_F1 DGAT1_3'UTR_R1	AGGAACTCGGAGTCCATCAC CTCCTCAGGGGCAGAAAAG		9	58	1.5	739	exon 14 - 3' UTR	EU178818.1
FASN_exon31_F1 FASN_exon31_R1	TGTGGGCTATGTGGTGAGG CATCTGGGTGGGTTTGGTC	g.14777C>T	11	59	2.5	400	partial intron 30 - partial exon 32	JN570752.2
SCD_5'UTR_F1 SCD_5'UTR_R1	AAATTCCTTCGGCCAATGAC TCTCACCTCTTGCAGCAA		22	58	1.5	527	partial promoter - exon 1	GQ904712.2
SCD_3'UTR_F SCD_3'UTR_R	GAAAGTATCCAAGGCTGCCG TGCTTCTTCTTTATCCTCAGCC		22	59	2	842	partial exon 6 - 3' UTR	GQ904712.2
For Genotyping								
ACAA2_3'UTR_F2 ACAA2_3'UTR_R2	ATTGACAACACAGCCTGAGGAGAGC TGGATTTTCAGTTCAGAAATGCTGCAT		23	60	2.5	247	partial exon 10 - 3' UTR	
DGAT1_3'UTR_F2 DGAT1_3'UTR_R2	ATGATGGCRCAGGTGAGCA AGGAGGCAGCTTYCACCAG		9	59	2	364	intron 16 - 3' UTR	
FASN_exon31_F2 FASN_exon31_R2	ACCATGTGGCAGGAAGTGTC AATGACCACTTTGCCGATGT		11	59	2.5	150	partial exon 31	
SCD_5'UTR_F2 SCD_5'UTR_R2	ATCCCCAGCACAGCAGGT CATTACTCGGAAGCTCTCACC		22	59	2.5	249	5' UTR – partial intron 1	

47 **Supplementary Table S2.** Descriptive statistics for milk fat content (%) and milk fatty acid (FA)
 48 composition (% , wt/wt fat)

Trait	Mean	Range		Std. Deviation	CV (%)
		Minimum	Maximum		
Milk fat %	5.27	2.69	8.02	0.91	17.28
Saturated Fatty Acids					51
C4:0	3.27	1.18	11.32	1.56	47.63
C5:0	0.04	0.01	0.12	0.02	51.54
C6:0	3.18	0.93	10.73	1.56	48.98
C7:0	0.07	0.01	0.32	0.04	65.53
C8:0	3.32	0.86	10.47	1.52	45.69
C9:0	0.11	0.01	0.31	0.05	48.54
C10:0	9.03	1.69	27.85	4.27	47.27
C11:0	0.13	0.02	0.47	0.07	52.79
C12:0	5.47	1.90	11.60	1.49	27.25
C13:0	0.12	0.02	0.58	0.07	62.28
<i>iso</i> C14:0	0.10	0.02	0.29	0.05	45.56
C14:0	11.20	5.95	15.33	1.56	13.95
<i>iso</i> C15:0	0.23	0.04	0.59	0.10	43.57
<i>anteiso</i> C15:0	0.46	0.04	1.44	0.19	40.68
C15:0	1.03	0.16	2.47	0.35	34.58
<i>iso</i> C16:0	0.28	0.02	2.90	0.24	86.42
C16:0	24.96	11.04	35.18	3.74	14.99
<i>iso</i> C17:0	0.35	0.03	0.76	0.15	42.38
<i>anteiso</i> C17:0	0.49	0.03	1.55	0.32	64.93
C17:0	0.57	0.04	1.43	0.23	40.99
C18:0	8.70	1.63	21.64	3.25	37.32
C20:0	0.30	0.03	0.98	0.22	73.61
C22:0	0.14	0.03	0.31	0.07	49.05
Monounsaturated Fatty Acids					62
C10:1	0.39	0.06	0.93	0.14	36.27
C12:1 <i>cis</i> -9	0.12	0.01	0.37	0.06	50.00
C14:1 <i>cis</i> -9	0.24	0.003	0.88	0.14	58.42
C16:1 <i>cis</i> -9	0.92	0.06	3.38	0.55	59.23
C16:1 <i>cis</i> -7	0.44	0.02	1.23	0.29	64.61
C17:1 <i>cis</i> -9	0.21	0.02	0.66	0.11	53.09
C18:1 <i>cis</i> -9	18.71	3.67	35.96	5.23	27.65
C18:1 <i>trans</i> -11	0.96	0.03	3.00	0.61	63.47
C18:1 <i>cis</i> -12 or <i>cis</i> -13	0.45	0.06	1.33	0.28	63.68
C20:1 <i>cis</i> -9	0.16	0.02	0.58	0.12	73.87
Polyunsaturated Fatty Acids					67
C18:2 other	0.15	0.02	0.52	0.09	62.49
C18:2 <i>cis</i> -9, <i>cis</i> -12	2.72	0.29	6.67	1.10	40.38
C18:2 <i>trans</i> -10, <i>cis</i> -12 CLA	0.17	0.01	0.51	0.11	65.98
C18:2 <i>cis</i> -9, <i>trans</i> -11 CLA	0.56	0.05	1.61	0.31	55.54
C18:3 <i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12	0.08	0.01	0.25	0.05	53.60
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.27	0.03	0.73	0.13	46.03
C20:4 <i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14	0.21	0.03	0.52	0.10	46.70
Fatty acid groups					
SFA ¹	72.92	51.89	93.81	7.21	9.88
UFA ²	26.28	6.16	47.32	6.88	26.20
MUFA ³	22.43	5.87	39.83	5.70	25.41
PUFA ⁴	3.85	0.29	9.03	1.54	39.88
SCFA ⁵	19.35	5.28	58.60	8.76	45.26
Mammary origin FA ⁶	38.43	15.42	80.03	10.53	27.63
MCFA ⁷	17.20	7.25	25.6	2.87	16.70
LCFA ⁸	60.77	19.97	82.6	10.32	17.28
Odd chain FA ⁹	3.32	0.43	7.01	1.16	34.98
ω 6/ ω 3 ¹⁰	12.03	0.28	64.37	6.68	55.74
AI ¹¹	3.19	1.10	13.09	1.39	43.40
EI ¹²	0.52	0.26	0.75	0.08	15.07

- 77 ¹Saturated fatty acids
78 ²Unsaturated fatty acids
79 ³Monounsaturated fatty acids
80 ⁴Polyunsaturated fatty acids
81 ⁵Short chain fatty acids (SFA and UFA from C4 to C10)
82 ⁶Mammary origin fatty acids (SFA and UFA from C4 to C15)
83 ⁷Medium chain fatty acids (SFA and UFA from C12 to C14)
84 ⁸Long chain fatty acids (SFA and UFA from C16 to C24)
85 ⁹Odd chain fatty acids (C11:0 + C13:0 + C15:0 + C15:1 + C17:0 + C17:1)
86 ¹⁰ $\omega 6/\omega 3$ (C18:2 *cis*-9, *cis*-12 + C18:3 *cis*-6, *cis*-9, *cis*-12 + C20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14)/ C18:3 *cis*-9, *cis*-12, *cis*-15
87 ¹¹Atherogenic index (C12:0 + 4*(C14:0) + C16:0)/(MUFA + PUFA)
88 ¹²Elongation index (C18:0 + C18:1)/(C16:0 + C16:1 + C18:0 + C18:1)

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91 **Supplementary Table S3.** Genotypic and allelic frequencies of identified SNPs of the *ACAA2* and *FASN*
92 genes

SNP	Genotypic frequencies	Allelic frequencies	HW equilibrium
<i>ACAA2</i> 3'UTR g.2982T>C	CC:0.20, CT:0.55, TT:0.25	C:0.47, T:0.53	$P=0.053$
<i>FASN</i> exon 31 g.14777C>T	CC:0.46, CT:0.44, TT:0.10	C:0.67, T:0.33	$P=0.478$

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