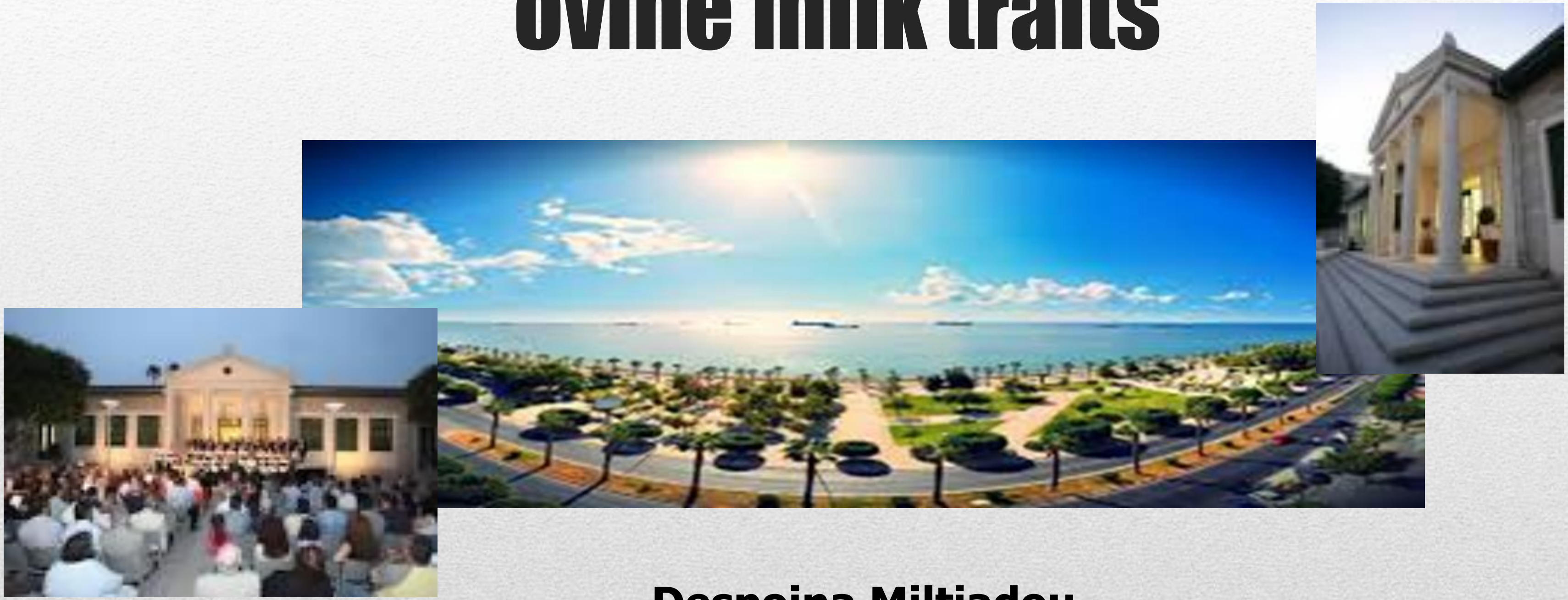
The putative role of ACAA2 in ovine milk traits



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Summary

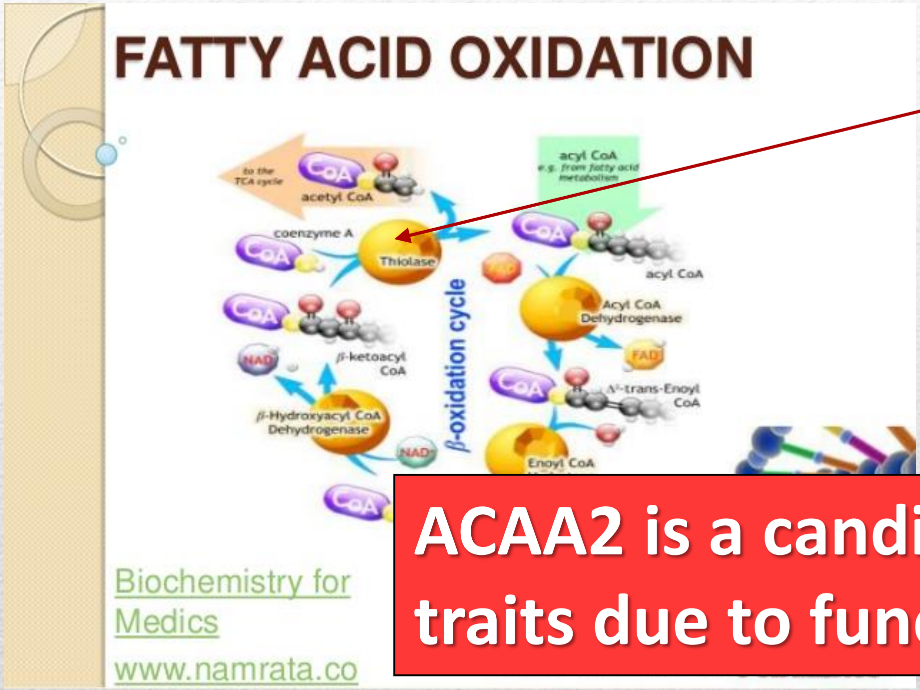
In the next 15 minutes ….

- Working hypothesis
- Association of *ACAA2* polymorphism with
  - milk traits
    - ➢ in a closed nucleus
    - ➢ in an extended population

  - with FA profile
- Functional *ACAA2* study
- Conclusions

Chios sheep

ACAA2: Acetyl-CoA acyl transferase 2



FATTY ACID OXIDATION

Biochemistry for Medics

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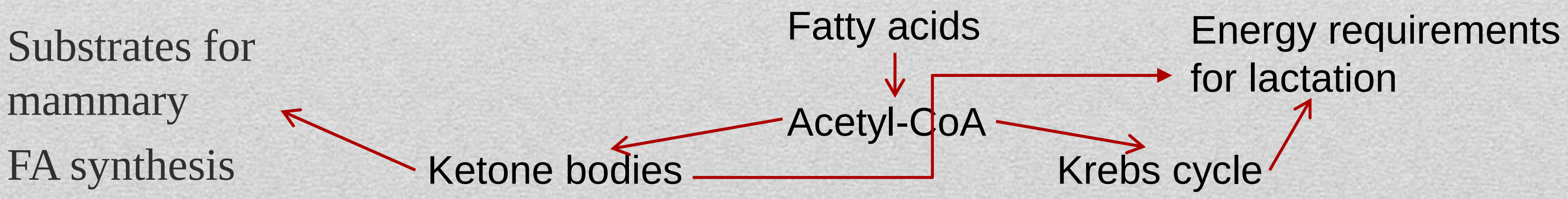
ACAA2 catalyses the last step of mitochondrial fatty acid oxidation

ACAA2 is a candidate gene for milk traits due to function



Fatty acids

Acetyl-CoA

Energy requirements for lactation

Ketone bodies

Krebs cycle

Substrates for mammary FA synthesis

Working hypothesis

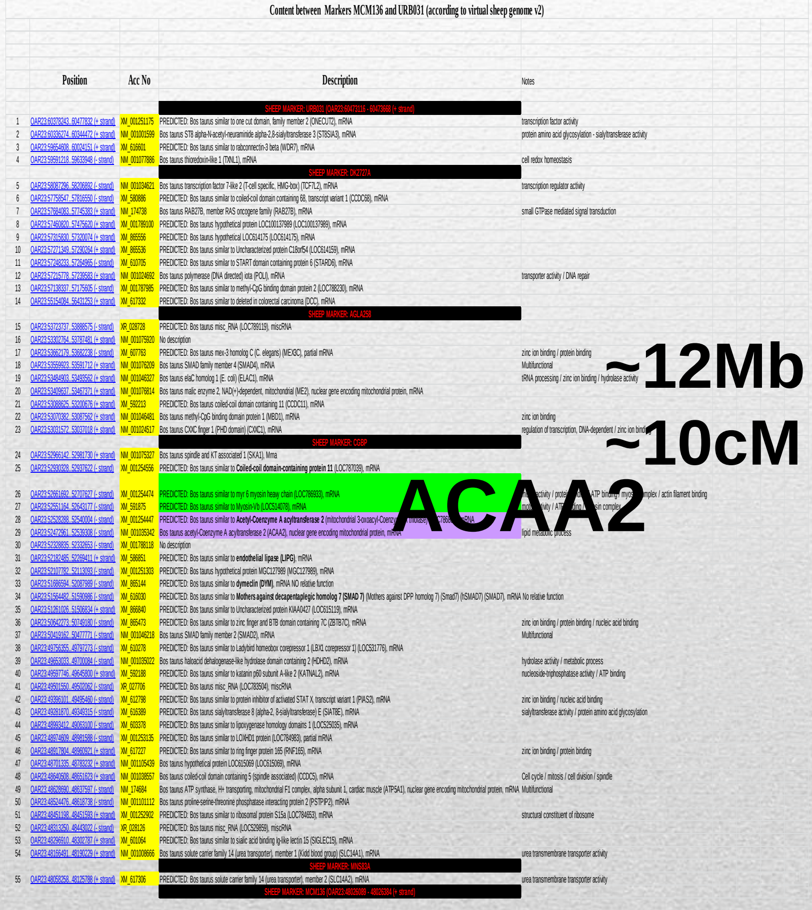
ACAA2

QTL for milk, fat and protein yield on ovine chromosome 23

QTL located around the middle of the region within markers MCM136 and URB031

ACAA2 is a candidate gene due to chromosomal location

Ovine chromosome OAR23



Content between Markers MCM136 and URB031 (according to virtual sheep genome v2)

	Position	Acc No	Description	Notes
			SHEEP MARKER: URB031 (OAR23:60473116 - 60473668 (+ strand)	
1	OAR23:60378243..60477832 (+ strand)	XM_001251175	PREDICTED: Bos taurus similar to one cut domain, family member 2 (ONECUT2), mRNA	transcription factor activity
2	OAR23:60336274..60344472 (+ strand)	NM_001001599	Bos taurus ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3 (ST8SIA3), mRNA	protein amino acid glycosylation - sialyltransferase activity
3	OAR23:59654608..60024151 (+ strand)	XM_616601	PREDICTED: Bos taurus similar to rabconnectin-3 beta (WDR7), mRNA	
4	OAR23:59591218..59633948 (- strand)	NM_001077886	Bos taurus thioredoxin-like 1 (TXNL1), mRNA	cell redox homeostasis
			SHEEP MARKER: DK2727A	
5	OAR23:58087296..58206892 (- strand)	NM_001034621	Bos taurus transcription factor 7-like 2 (T-cell specific, HMG-box) (TCF7L2), mRNA	transcription regulator activity
6	OAR23:57758547..57816550 (- strand)	XM_580886	PREDICTED: Bos taurus similar to coiled-coil domain containing 68, transcript variant 1 (CCDC68), mRNA	
7	OAR23:57684083..57745383 (+ strand)	NM_174738	Bos taurus RAB27B, member RAS oncogene family (RAB27B), mRNA	small GTPase mediated signal transduction
8	OAR23:57460820..57475620 (+ strand)	XM_001789100	PREDICTED: Bos taurus hypothetical protein LOC100137989 (LOC100137989), mRNA	
9	OAR23:57315830..57320074 (+ strand)	XM_865556	PREDICTED: Bos taurus hypothetical LOC614175 (LOC614175), mRNA	
10	OAR23:57271349..57290264 (+ strand)	XM_865536	PREDICTED: Bos taurus similar to Uncharacterized protein C18orf54 (LOC614159), mRNA	
11	OAR23:57248233..57264965 (- strand)	XM_610705	PREDICTED: Bos taurus similar to START domain containing protein 6 (STARD6), mRNA	
12	OAR23:57215778..57239583 (+ strand)	NM_001024692	Bos taurus polymerase (DNA directed) iota (POLI), mRNA	transporter activity / DNA repair
13	OAR23:57138337..57175605 (- strand)	XM_001787985	PREDICTED: Bos taurus similar to methyl-CpG binding domain protein 2 (LOC788230), mRNA	
14	OAR23:55154084..56431253 (+ strand)	XM_617332	PREDICTED: Bos taurus similar to deleted in colorectal carcinoma (DCC), mRNA	
			SHEEP MARKER: AGLA258	
15	OAR23:53723737..53888575 (- strand)	XR_028728	PREDICTED: Bos taurus misc_RNA (LOC789119), miscRNA	
16	OAR23:53302764..53787481 (+ strand)	NM_001075920	No description	
17	OAR23:53662179..53682238 (- strand)	XM_607763	PREDICTED: Bos taurus mex-3 homolog C (C. elegans) (MEX3C), partial mRNA	zinc ion binding / protein binding
18	OAR23:53559923..53591712 (+ strand)	NM_001076209	Bos taurus SMAD family member 4 (SMAD4), mRNA	Multifunctional
19	OAR23:53484903..53493562 (+ strand)	NM_001046327	Bos taurus elaC homolog 1 (E. coli) (ELAC1), mRNA	tRNA processing / zinc ion binding / hydrolase activity
20	OAR23:53409637..53467371 (+ strand)	NM_001076814	Bos taurus malic enzyme 2, NAD(+)-dependent, mitochondrial (ME2), nuclear gene encoding mitochondrial protein, mRNA	
21	OAR23:53088625..53200676 (+ strand)	XM_592213	PREDICTED: Bos taurus coiled-coil domain containing 11 (CCDC11), mRNA	
22	OAR23:53070382..53087562 (+ strand)	NM_001046481	Bos taurus methyl-CpG binding domain protein 1 (MBD1), mRNA	zinc ion binding
23	OAR23:53031572..53037018 (+ strand)	NM_001024517	Bos taurus CXXC finger 1 (PHD domain) (CXXC1), mRNA	regulation of transcription, DNA-dependent / zinc ion binding
			SHEEP MARKER: CGBP	
24	OAR23:52966142..52981730 (+ strand)	NM_001075327	Bos taurus spindle and KT associated 1 (SKA1), Mrna	
25	OAR23:52930328..52937622 (- strand)	XM_001254556	PREDICTED: Bos taurus similar to **Coiled-coil domain-containing protein 11** (LOC787039), mRNA	
26	OAR23:52661692..52707627 (- strand)	XM_001254474	PREDICTED: Bos taurus similar to myr 6 myosin heavy chain (LOC786933), mRNA	m[illegible] activity / protei[illegible]nding / ATP binding / myos[illegible]mplex / actin filament binding
27	OAR23:52551164..52643177 (- strand)	XM_591875	PREDICTED: Bos taurus similar to Myosin-Vb (LOC514078), mRNA	moto[illegible]tivity / AT[illegible]ding / [illegible]sin complex
28	OAR23:52528288..52540004 (- strand)	XM_001254447	PREDICTED: Bos taurus similar to **Acetyl-Coenzyme A acyltransferase 2** (mitochondrial 3-oxoacyl-Coenzy[illegible] thiolase) [illegible]C7868[illegible] mRNA	
29	OAR23:52472961..52539308 (- strand)	NM_001035342	Bos taurus acetyl-Coenzyme A acyltransferase 2 (ACAA2), nuclear gene encoding mitochondrial protein, mRNA	lipid metabolic process
30	OAR23:52328835..52332653 (- strand)	XM_001788118	No description	
31	OAR23:52182485..52269411 (+ strand)	XM_586851	PREDICTED: Bos taurus similar to **endothelial lipase (LIPG)**, mRNA	
32	OAR23:52107782..52113093 (- strand)	XM_001251303	PREDICTED: Bos taurus hypothetical protein MGC127989 (MGC127989), mRNA	
33	OAR23:51686594..52087989 (- strand)	XM_865144	PREDICTED: Bos taurus similar to **dymeclin (DYM)**, mRNA NO relative function	
34	OAR23:51564482..51590986 (- strand)	XM_616030	PREDICTED: Bos taurus similar to **Mothers against decapentaplegic homolog 7 (SMAD 7)** (Mothers against DPP homolog 7) (Smad7) (hSMAD7) (SMAD7), mRNA	No relative function
35	OAR23:51261026..51506834 (+ strand)	XM_866840	PREDICTED: Bos taurus similar to Uncharacterized protein KIAA0427 (LOC615119), mRNA	
36	OAR23:50642273..50749180 (- strand)	XM_865473	PREDICTED: Bos taurus similar to zinc finger and BTB domain containing 7C (ZBTB7C), mRNA	zinc ion binding / protein binding / nucleic acid binding
37	OAR23:50419162..50477771 (- strand)	NM_001046218	Bos taurus SMAD family member 2 (SMAD2), mRNA	Multifunctional
38	OAR23:49756355..49797273 (- strand)	XM_610278	PREDICTED: Bos taurus similar to Ladybird homeobox corepressor 1 (LBX1 corepressor 1) (LOC531776), mRNA	
39	OAR23:49653033..49700084 (- strand)	NM_001035022	Bos taurus haloacid dehalogenase-like hydrolase domain containing 2 (HDHD2), mRNA	hydrolase activity / metabolic process
40	OAR23:49597746..49645800 (+ strand)	XM_592188	PREDICTED: Bos taurus similar to katanin p60 subunit A-like 2 (KATNAL2), mRNA	nucleoside-triphosphatase activity / ATP binding
41	OAR23:49501550..49502062 (- strand)	XR_027706	PREDICTED: Bos taurus misc_RNA (LOC783504), miscRNA	
42	OAR23:49396101..49495460 (- strand)	XM_612798	PREDICTED: Bos taurus similar to protein inhibitor of activated STAT X, transcript variant 1 (PIAS2), mRNA	zinc ion binding / nucleic acid binding
43	OAR23:49281870..49349315 (- strand)	XM_616389	PREDICTED: Bos taurus sialyltransferase 8 (alpha-2, 8-sialyltransferase) E (SIAT8E), mRNA	sialyltransferase activity / protein amino acid glycosylation
44	OAR23:48993412..49063100 (- strand)	XM_603378	PREDICTED: Bos taurus similar to lipoxygenase homology domains 1 (LOC525035), mRNA	
45	OAR23:48974609..48981588 (- strand)	XM_001253135	PREDICTED: Bos taurus similar to LOXHD1 protein (LOC784983), partial mRNA	
46	OAR23:48917804..48960921 (+ strand)	XM_617227	PREDICTED: Bos taurus similar to ring finger protein 165 (RNF165), mRNA	zinc ion binding / protein binding
47	OAR23:48701335..48783232 (+ strand)	NM_001105439	Bos taurus hypothetical protein LOC615069 (LOC615069), mRNA	
48	OAR23:48640508..48651623 (+ strand)	NM_001038557	Bos taurus coiled-coil domain containing 5 (spindle associated) (CCDC5), mRNA	Cell cycle / mitosis / cell division / spindle
49	OAR23:48628690..48637597 (- strand)	NM_174684	Bos taurus ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1), nuclear gene encoding mitochondrial protein, mRNA	Multifunctional
50	OAR23:48524476..48618738 (- strand)	NM_001101112	Bos taurus proline-serine-threonine phosphatase interacting protein 2 (PSTPIP2), mRNA	
51	OAR23:48451198..48451593 (+ strand)	XM_001252902	PREDICTED: Bos taurus similar to ribosomal protein S15a (LOC784653), mRNA	structural constituent of ribosome
52	OAR23:48313250..48443022 (- strand)	XR_028126	PREDICTED: Bos taurus misc_RNA (LOC529859), miscRNA	
53	OAR23:48296910..48302787 (+ strand)	XM_601064	PREDICTED: Bos taurus similar to sialic acid binding Ig-like lectin 15 (SIGLEC15), mRNA	
54	OAR23:48166491..48190229 (+ strand)	NM_001008666	Bos taurus solute carrier family 14 (urea transporter), member 1 (Kidd blood group) (SLC14A1), mRNA	urea transmembrane transporter activity
			SHEEP MARKER: MNS83A	
55	OAR23:48058258..48125788 (+ strand)	XM_617306	PREDICTED: Bos taurus solute carrier family 14 (urea transporter), member 2 (SLC14A2), mRNA	urea transmembrane transporter activity
			SHEEP MARKER: MCM136 (OAR23:48026089 - 48026384 (+ strand)	

~12Mb

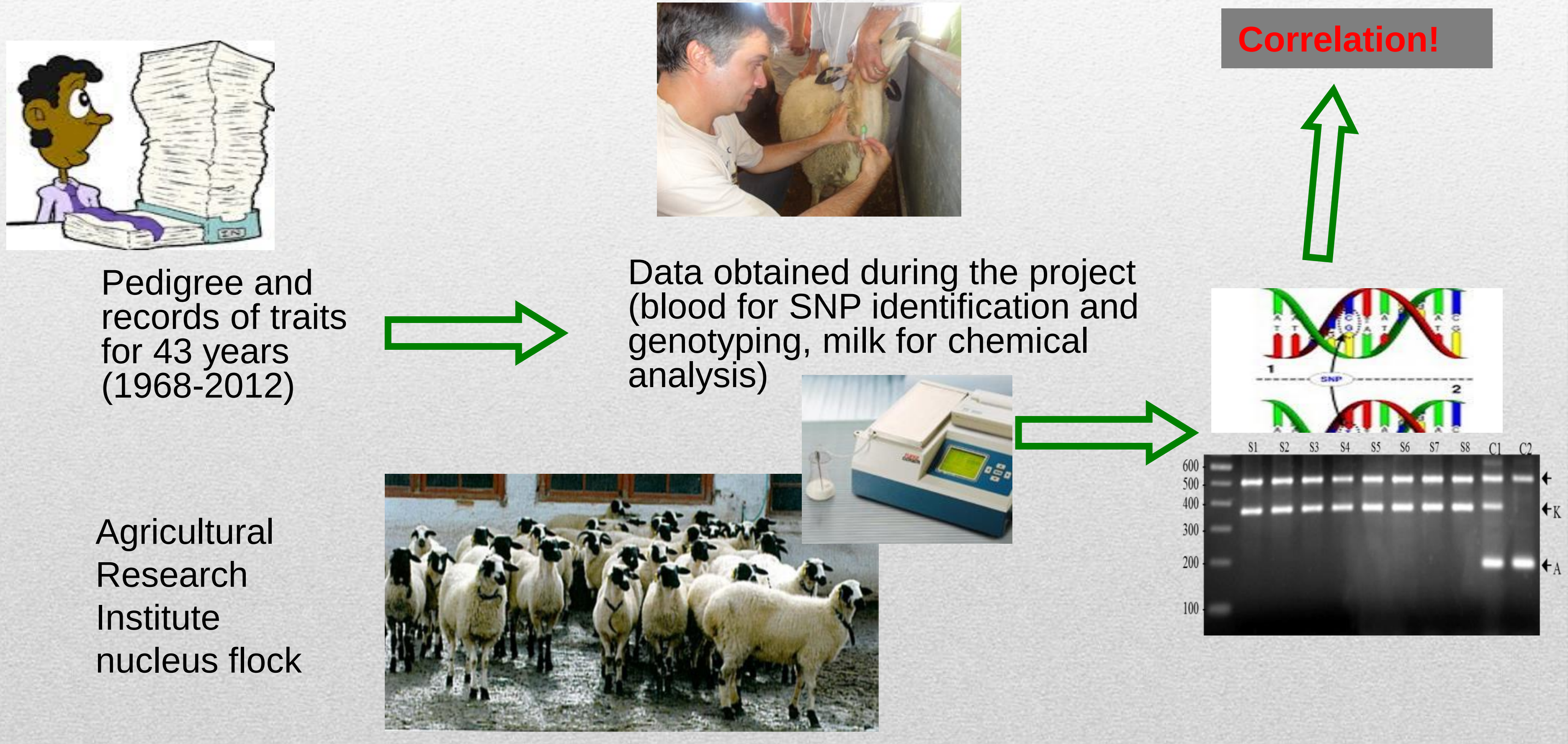
~10cM

ACAA2

Working hypothesis

(whole genome QTL identification study in sheep; Gutierez-Gill, 2009)

Project title:
Use of molecular and quantitative genetics for the improvement of Chios sheep



Pedigree and records of traits for 43 years (1968-2012)

Data obtained during the project (blood for SNP identification and genotyping, milk for chemical analysis)

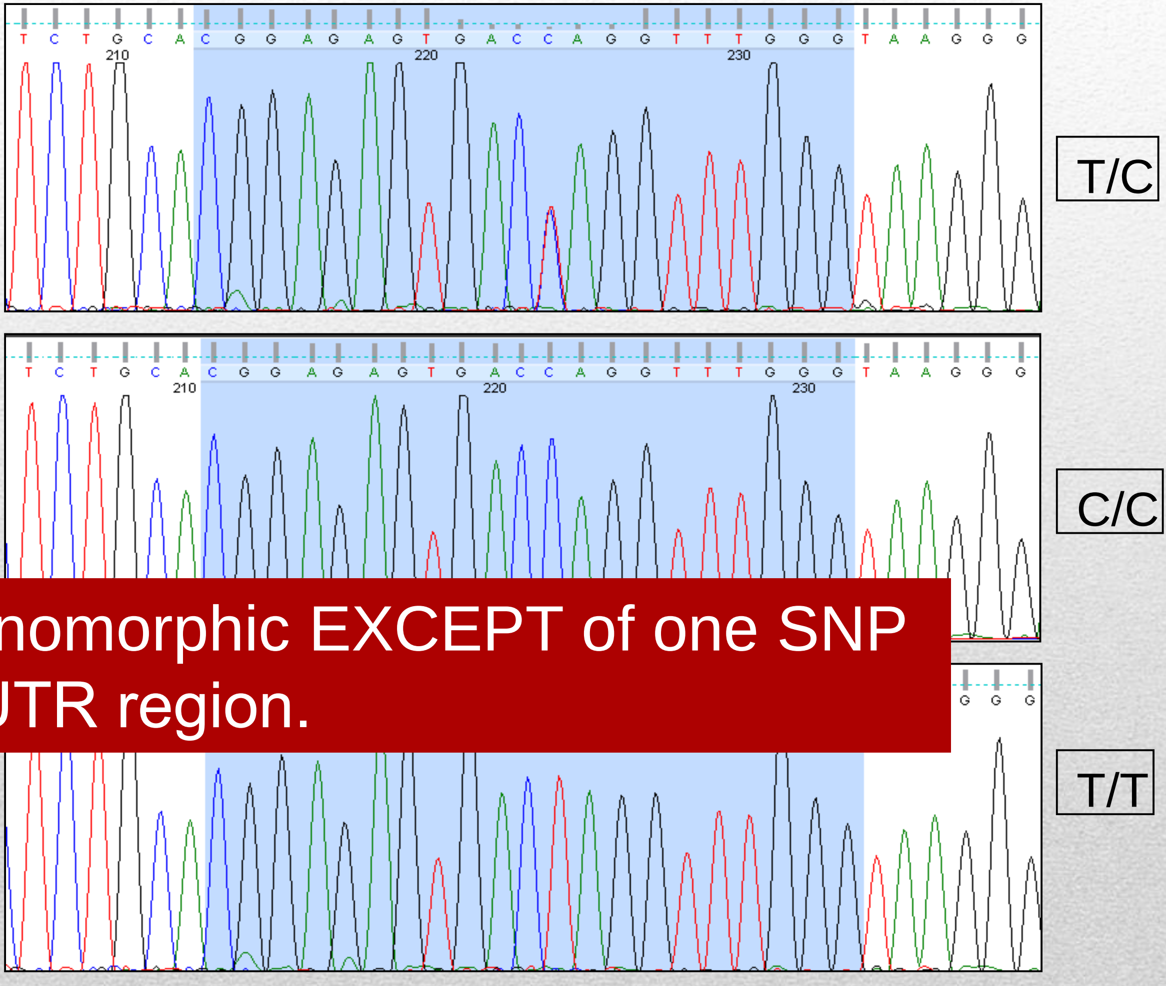
Correlation!

Agricultural Research Institute nucleus flock

Work flow of the project

ACAA2

Sequence based SNP identification



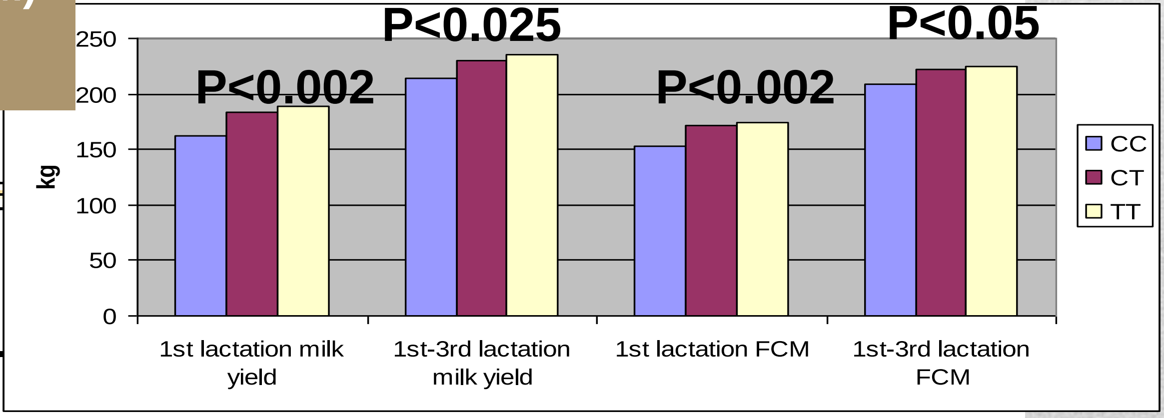
All exons were monomorphic EXCEPT of one SNP identified in the 3’UTR region.

SNP identification

ACAA2 IS SIGNIFICANTLY ASSOCIATED WITH MILK YIELD AND FCM

- 318 genotyped ewes
- Offspring from 104 sires
- 717 records for
  - milk yield
  - fat corrected milk (FCM)
  - fat percentage

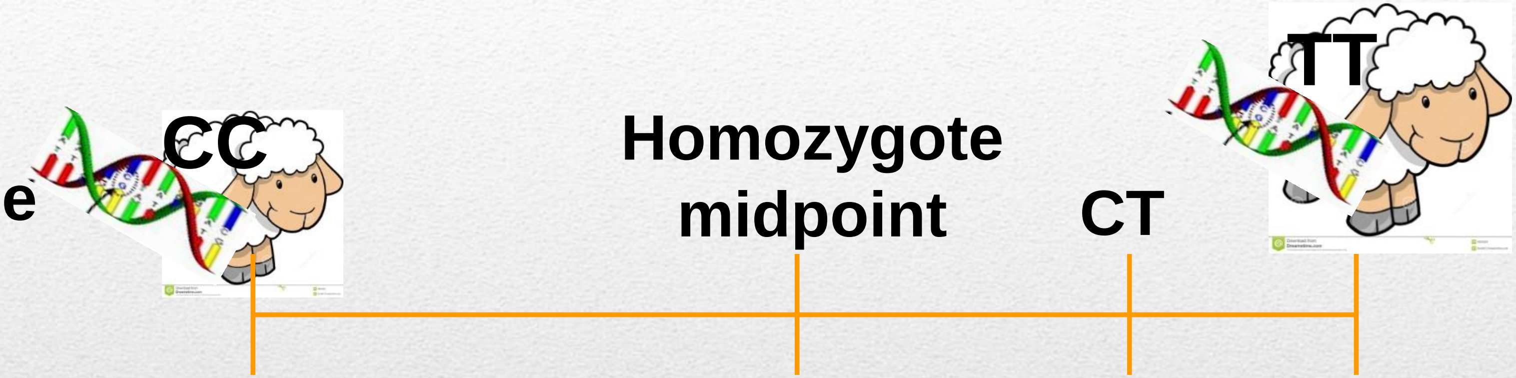
	ACAA2 genotype			*Allelic frequency*	
ls	CC	CT	TT	C-allele	T-allele
Hardy-Weinberg Prediction 343					



ACAA2 association with milk traits

ACAA2

Genotype means for first lactation milk yield



Genotype Class	CC	Homozygote midpoint	CT	TT
Class Mean (kg)	161.7	175.1	182.9	188.4

- Ewes with the T … hat ewes with CC g
- Partial dominan

THE GENE EFFECT COULD BE REGARDED AS A MAJOR GENE EFFECT
WITH HOMOZYGOUS DIFFERING BY 0.5SD
AND
THE 3'UTR SNP EXPLAINED 10% OF THE ADDITIVE GENETIC VARIANCE

Orford M., Tzamaloukas O., Hadjipavlou G., Hatziplis D., Koumas A., Mavrogenis A., Papachristoforou C., **Miltiadou D.*** (2012) ***J. Dairy Sci.*** 95:3419–3427

ACAA2 association with milk traits

ACAA2 association with milk traits

Confirmed previous association of **ACAA2 with milk yield** in an extended population of Chios

ACAA2 is associated with milk protein %

	all lactations milk yield	1^{st} lactation milk yield	milk protein percentage	milk fat percentage	milk protein yield	milk fat yield
a^{1}±SE	6.81±2.95	10.61±3.56	-0.05±0.02	-0.03±0.03	0.23±0.18	0.28±0.17
P^{2}	0.021*	0.003*	0.003*	0.370	0.200	0.100
d^{3}±SE	8.67±3.53	13.02±4.26	0.02±0.02	0.04±0.04	0.41±0.21	0.46±0.21
P^{4}	0.014*	0.002*	0.41	0.322	0.048*	
% V_P due to SNP^{5}	0.62%	2.25%	1.68%	0.3	44%	

ACAA2 is NOT associated with milk fat% and is associated with fat yield

Dillution effect

Results from 742 animals from all sheep farms keeping da rus

Trait	all lactations milk yield^{1}	1^{st} lactation milk yield^{1}	milk protein percentage	milk fat percentage		mi yi
	Genotyped means±SE^{2}					
CC	245.23±4.44^{a}	170.21±5.28^{a}	5.29±0.02^{a}	5.19±0.05	8.78±0.25	12.87±0.26^{a}
CT	260.71±2.76^{c}	193.85±3.33^{d}	5.23±0.01^{c}	5.20±0.03	9.42±0.16	13.62±0.17^{d}
TT	258.85±3.88^{b}	191.44±4.77^{c}	5.20±0.02^{c}	5.14±0.04	9.23±0.25	13.44±0.23^{b}

PROJECT TITLE:
IMPROVEMENT OF LIPID FRACTION IN OVINE MILK AND HALLOUMI CHEESE, TOWARDS HEALTHIER LOCAL PRODUCTS

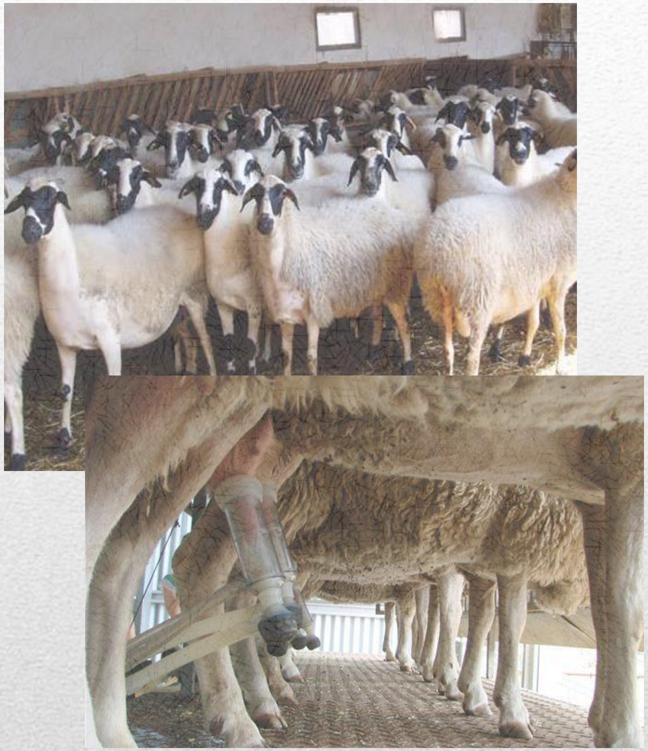
Collection of milk samples

4 Chios Farms → 1st 171 ewes
→ 2nd 118 ewes
→ 3rd 94 ewes
→ 4th 46 ewes

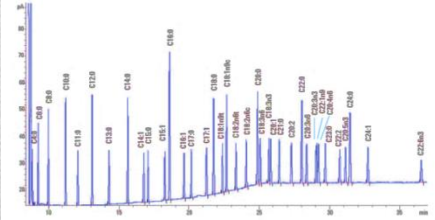
At least 3 samplings/animal

Total **429** Chios ewes

~**1300** milk samples

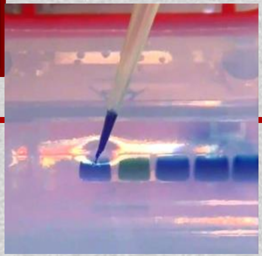


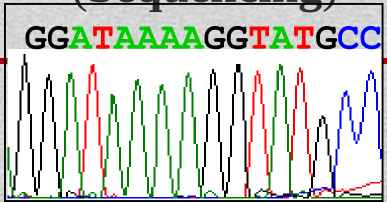
MILK → fat, protein, lactose
→ fame → fatty acid profile (**GC-MS**)



~**430** blood samples

→DNA extraction → Genotyping
(**Sequencing**)

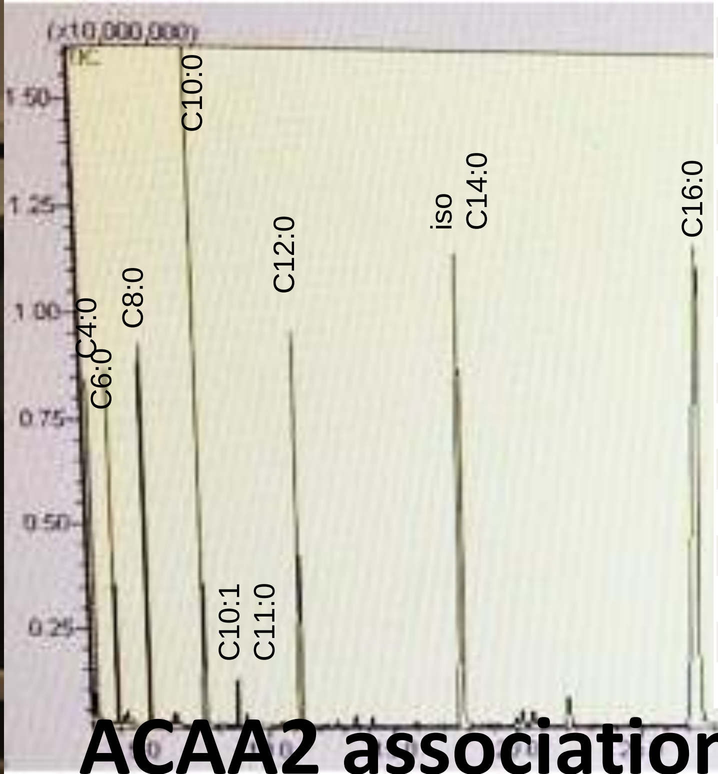




GCMS RESULTS

FAME	mean %w/w	sd	FAME	mean %w/w	sd
C4:0	3.27	1.56	C16:1 cis9	0.92	0.55
C5:0	0.04	0.02	iso C17:0	0.34	0.15
C6:0	3.18	1.56	C16:1 other	0.44	0.29
C7:0	0.07	0.04	anteiso C17:0	0.49	0.32
C8:0				0.57	0.23
C9:0				0.21	0.11
C10:0				8.70	3.25
C10:1				2.14	3.00
C11:0				18.70	5.23
C12:0				0.96	0.61
C12:1cis9				0.45	0.28
C13:0				0.14	0.09
iso C14:0				2.72	1.10
C14:0				0.17	0.11
iso C15:0				0.08	0.04
C14:1				0.27	0.13
ant C15:0				0.30	0.22
cis10 C15:1	0.07	0.04	Linoleic acid (9Z, 11E)	0.56	0.31
C15:0	1.03	0.35	cis-11-C20:1	0.16	0.12
iso C16	0.28	0.24	C20:4ω6	0.21	0.10
C16:0	24.96	3.74	C22:0	0.14	0.07
			cis_13_C22:1_ω9	0.15	0.08

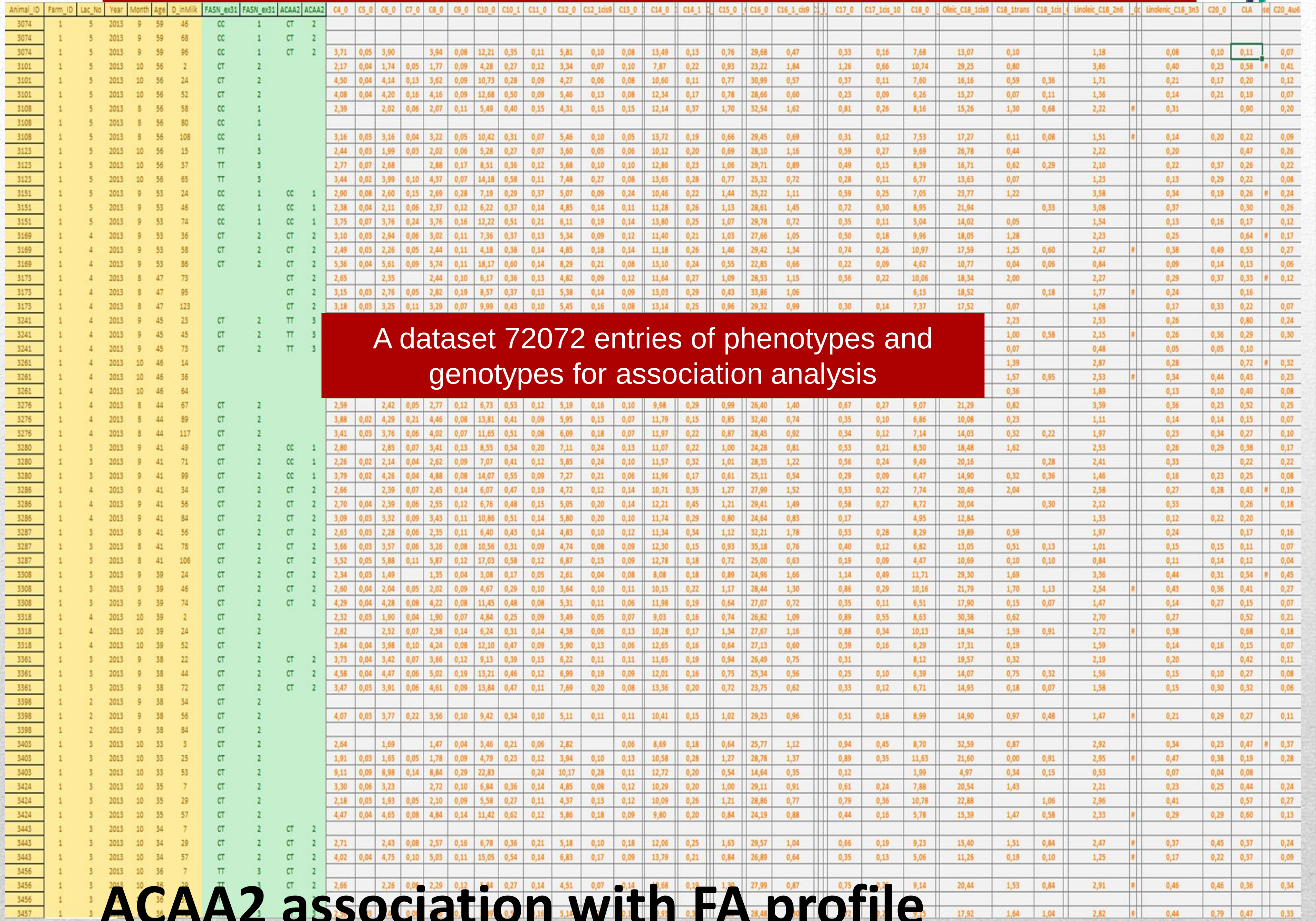
FAME	mean %w/w	sd
SFA	72.93	7.21
SCFA (4C-10C)	18.98	8.67
mammary origin C4-C14	35.96	10.70
MCFA (12C-14C)	16.86	2.83
LCFA(16C-24C)	36.00	5.24
MUFA	22.42	5.70
PUFA	3.85	1.54
UFA	26.25	6.88
C<16	64.81	9.89
C>16	34.39	9.55



ACAA2 association with FA profile

ACAA2 association with FA profile

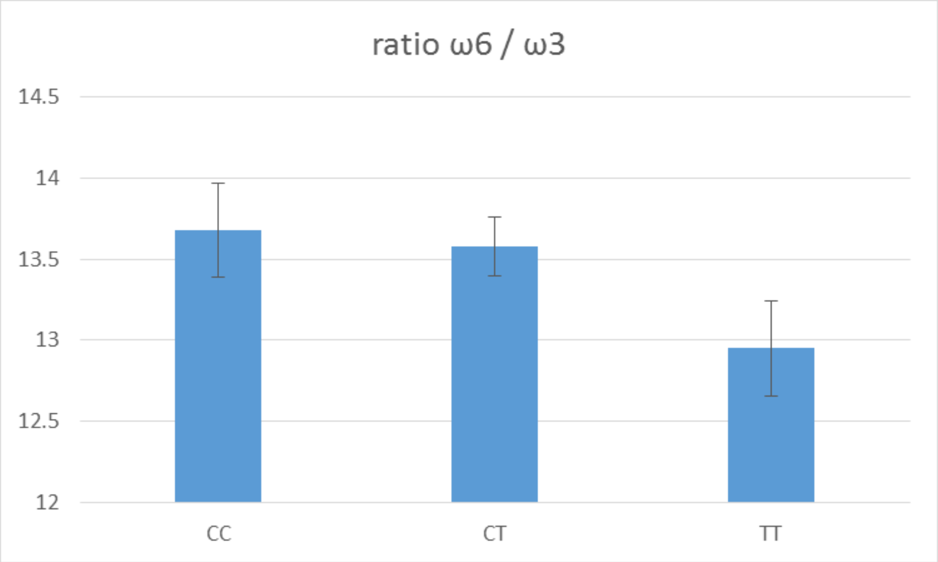
A dataset 72072 entries of phenotypes and genotypes for association analysis



Animal_ID	Farm_ID	Lac_No	Year	Month	Age	D_inMilk	FASN_ex31	FASN_ex31	ACAA2	ACAA2	C4_0	C5_0	C6_0	C7_0	C8_0	C9_0	C10_0	C10_1	C11_0	C12_0	C12_1cis9	C13_0	C14_0	C14_1	C15_0	C16_0	C16_1_cis9	C17_0	C17_1cis_10	C18_0	Oleic_C18_1cis9	C18_1trans	C18_1cis	Linoleic_C18_2n6		Linolenic_C18_3n3	C20_0	CLA		C20_4w6
3074	1	5	2013	9	59	46	CC	1	CT	2																														
3074	1	5	2013	9	59	68	CC	1	CT	2																														
3074	1	5	2013	9	59	96	CC	1	CT	2	3,71	0,05	3,90		3,94	0,08	12,21	0,35	0,11	5,81	0,10	0,08	13,49	0,13	0,76	29,68	0,47	0,33	0,16	7,68	13,07	0,10		1,18		0,08	0,10	0,11		0,07
3101	1	5	2013	10	56	2	CT	2			2,17	0,04	1,74	0,05	1,77	0,09	4,28	0,27	0,12	3,34	0,07	0,10	7,87	0,22	0,93	23,22	1,84	1,26	0,66	10,74	29,25	0,80		3,86		0,40	0,23	0,58	#	0,41
3101	1	5	2013	10	56	24	CT	2			4,50	0,04	4,14	0,13	3,62	0,09	10,73	0,28	0,09	4,27	0,06	0,08	10,60	0,11	0,77	30,99	0,57	0,37	0,11	7,60	16,16	0,59	0,36	1,71		0,21	0,17	0,20		0,12
3101	1	5	2013	10	56	52	CT	2			4,08	0,04	4,20	0,16	4,16	0,09	12,68	0,50	0,09	5,46	0,13	0,08	12,34	0,17	0,78	28,66	0,60	0,23	0,09	6,26	15,27	0,07	0,11	1,36		0,14	0,21	0,19		0,07
3108	1	5	2013	8	56	58	CC	1			2,39		2,02	0,06	2,07	0,11	5,49	0,40	0,15	4,31	0,15	0,15	12,14	0,37	1,70	32,54	1,62	0,81	0,26	8,16	15,26	1,30	0,68	2,22	#	0,31		0,90		0,20
3108	1	5	2013	8	56	80	CC	1																																
3108	1	5	2013	8	56	108	CC	1			3,16	0,03	3,16	0,04	3,22	0,05	10,42	0,31	0,07	5,46	0,10	0,05	13,72	0,19	0,66	29,45	0,69	0,31	0,12	7,53	17,27	0,11	0,08	1,51	#	0,14	0,20	0,22		0,09
3123	1	5	2013	10	56	15	TT	3			2,44	0,03	1,99	0,03	2,02	0,06	5,28	0,27	0,07	3,60	0,05	0,06	10,12	0,20	0,69	28,10	1,16	0,59	0,27	9,69	26,78	0,44		2,22		0,20		0,47		0,26
3123	1	5	2013	10	56	37	TT	3			2,77	0,07	2,68		2,88	0,17	8,51	0,36	0,12	5,68	0,10	0,10	12,86	0,23	1,06	29,71	0,89	0,49	0,15	8,39	16,71	0,62	0,29	2,10		0,22	0,37	0,26		0,22
3123	1	5	2013	10	56	65	TT	3			3,44	0,02	3,99	0,10	4,37	0,07	14,18	0,58	0,11	7,48	0,27	0,08	13,65	0,28	0,77	25,32	0,72	0,28	0,11	6,77	13,63	0,07		1,23		0,13	0,29	0,22		0,08
3151	1	5	2013	9	53	24	CC	1	CC	1	2,90	0,08	2,60	0,15	2,69	0,28	7,19	0,29	0,37	5,07	0,09	0,24	10,46	0,22	1,44	25,22	1,11	0,59	0,25	7,05	23,77	1,22		3,58		0,34	0,19	0,26	#	0,24
3151	1	5	2013	9	53	46	CC	1	CC	1	2,38	0,04	2,11	0,06	2,37	0,12	6,22	0,37	0,14	4,85	0,14	0,11	11,28	0,26	1,13	28,61	1,45	0,72	0,30	8,95	21,94		0,33	3,08		0,37		0,30		0,26
3151	1	5	2013	9	53	74	CC	1	CC	1	3,75	0,07	3,76	0,24	3,76	0,16	12,22	0,51	0,21	6,11	0,19	0,14	13,80	0,25	1,07	29,78	0,72	0,35	0,11	5,04	14,02	0,05		1,54		0,13	0,16	0,17		0,12
3169	1	4	2013	9	53	36	CT	2	CT	2	3,10	0,03	2,94	0,06	3,02	0,11	7,36	0,37	0,13	5,34	0,09	0,12	11,40	0,21	1,03	27,66	1,05	0,50	0,18	9,96	18,05	1,28		2,23		0,25		0,64	#	0,17
3169	1	4	2013	9	53	58	CT	2	CT	2	2,49	0,03	2,26	0,05	2,44	0,11	4,18	0,38	0,14	4,85	0,18	0,14	11,18	0,26	1,46	29,42	1,34	0,74	0,26	10,97	17,59	1,25	0,60	2,47	#	0,38	0,49	0,53		0,27
3169	1	4	2013	9	53	86	CT	2	CT	2	5,36	0,04	5,61	0,09	5,74	0,11	18,17	0,60	0,14	8,29	0,21	0,08	13,10	0,24	0,55	22,85	0,66	0,22	0,09	4,62	10,77	0,04	0,06	0,84		0,09	0,14	0,13		0,06
3173	1	4	2013	8	47	73			CT	2	2,65		2,35		2,44	0,10	6,17	0,36	0,13	4,82	0,09	0,12	11,64	0,27	1,09	28,53	1,15	0,56	0,22	10,06	18,34	2,00		2,27		0,29	0,37	0,33	#	0,12
3173	1	4	2013	8	47	95			CT	2	3,15	0,03	2,76	0,05	2,82	0,19	8,57	0,37	0,13	5,38	0,14	0,09	13,03	0,29	0,43	33,86	1,06			6,15	18,52		0,18	1,77	#	0,24		0,16		
3173	1	4	2013	8	47	123			CT	2	3,18	0,03	3,25	0,11	3,29	0,07	9,99	0,43	0,10	5,45	0,16	0,08	13,14	0,25	0,96	29,32	0,99	0,30	0,14	7,37	17,52	0,07		1,08		0,17	0,33	0,22		0,07
3241	1	4	2013	9	45	23	CT	2	TT	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2,23		2,53		0,26		0,80		0,24
3241	1	4	2013	9	45	45	CT	2	TT	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1,00	0,58	2,15	#	0,26	0,36	0,29		0,30
3241	1	4	2013	9	45	73	CT	2	TT	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0,07		0,48		0,05	0,05	0,10		
3261	1	4	2013	10	46	14					[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1,39		2,87		0,28		0,72	#	0,32
3261	1	4	2013	10	46	36					[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1,57	0,95	2,53	#	0,34	0,44	0,43		0,23
3261	1	4	2013	10	46	64					[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0,36		1,89		0,13	0,10	0,40		0,08
3276	1	4	2013	8	44	67	CT	2			2,59		2,42	0,05	2,77	0,12	6,73	0,53	0,12	5,19	0,16	0,10	9,98	0,29	0,99	26,40	1,40	0,67	0,27	9,07	21,29	0,82		3,39		0,36	0,23	0,52		0,25
3276	1	4	2013	8	44	89	CT	2			3,88	0,02	4,29	0,21	4,46	0,08	13,81	0,41	0,09	5,95	0,13	0,07	11,79	0,15	0,85	32,40	0,74	0,35	0,10	6,86	10,08	0,23		1,11		0,14	0,14	0,15		0,07
3276	1	4	2013	8	44	117	CT	2			3,41	0,03	3,76	0,06	4,02	0,07	11,65	0,51	0,08	6,09	0,18	0,07	11,97	0,22	0,87	28,45	0,92	0,34	0,12	7,14	14,03	0,32	0,22	1,97		0,23	0,34	0,27		0,10
3280	1	3	2013	9	41	49	CT	2	CC	1	2,80		2,85	0,07	3,41	0,13	8,55	0,54	0,20	7,11	0,24	0,13	11,07	0,22	1,00	24,28	0,81	0,53	0,21	8,50	18,48	1,62		2,53		0,26	0,29	0,38		0,17
3280	1	3	2013	9	41	71	CT	2	CC	1	2,26	0,02	2,14	0,04	2,62	0,09	7,07	0,41	0,12	5,85	0,24	0,10	11,57	0,32	1,01	28,35	1,22	0,56	0,24	9,49	20,16		0,28	2,41		0,33		0,22		0,22
3280	1	3	2013	9	41	99	CT	2	CC	1	3,79	0,02	4,26	0,04	4,88	0,08	14,07	0,55	0,09	7,27	0,21	0,06	11,96	0,17	0,61	25,11	0,54	0,29	0,09	6,47	14,90	0,32	0,36	1,46		0,16	0,23	0,25		0,08
3286	1	4	2013	9	41	34	CT	2	CT	2	2,66		2,39	0,07	2,45	0,14	6,07	0,47	0,19	4,72	0,12	0,14	10,71	0,35	1,27	27,99	1,52	0,53	0,22	7,74	20,49	2,04		2,58		0,27	0,28	0,43	#	0,19
3286	1	4	2013	9	41	56	CT	2	CT	2	2,70	0,04	2,39	0,06	2,55	0,12	6,76	0,48	0,15	5,05	0,20	0,14	12,21	0,45	1,21	29,41	1,49	0,58	0,27	8,72	20,04		0,30	2,12		0,33		0,26		0,18
3286	1	4	2013	9	41	84	CT	2	CT	2	3,09	0,03	3,32	0,09	3,43	0,11	10,86	0,51	0,14	5,80	0,20	0,10	11,74	0,29	0,80	24,64	0,83	0,17		4,95	12,84			1,33		0,12	0,22	0,20		
3287	1	3	2013	8	41	56	CT	2	CT	2	2,63	0,03	2,28	0,06	2,35	0,11	6,40	0,43	0,14	4,83	0,10	0,12	11,34	0,34	1,12	32,21	1,78	0,53	0,28	8,29	19,89	0,59		1,97		0,24		0,17		0,16
3287	1	3	2013	8	41	78	CT	2	CT	2	3,66	0,03	3,57	0,06	3,26	0,08	10,56	0,31	0,09	4,74	0,08	0,09	12,30	0,15	0,93	35,18	0,76	0,40	0,12	6,82	13,05	0,51	0,13	1,01		0,15	0,15	0,11		0,07
3287	1	3	2013	8	41	106	CT	2	CT	2	5,52	0,05	5,88	0,11	5,87	0,12	17,03	0,58	0,12	6,87	0,15	0,09	12,78	0,18	0,72	25,00	0,63	0,19	0,09	4,47	10,69	0,10	0,10	0,84		0,11	0,14	0,12		0,04
3308	1	3	2013	9	39	24	CT	2	CT	2	2,34	0,03	1,49		1,35	0,04	3,08	0,17	0,05	2,61	0,04	0,08	8,08	0,18	0,89	24,96	1,66	1,14	0,49	11,71	29,30	1,69		3,36		0,44	0,31	0,54	#	0,45
3308	1	3	2013	9	39	46	CT	2	CT	2	2,60	0,04	2,04	0,05	2,02	0,09	4,67	0,29	0,10	3,64	0,10	0,11	10,15	0,22	1,17	28,44	1,30	0,86	0,29	10,16	21,79	1,70	1,13	2,54	#	0,43	0,36	0,41		0,27
3308	1	3	2013	9	39	74	CT	2	CT	2	4,29	0,04	4,28	0,08	4,22	0,08	11,45	0,48	0,08	5,31	0,11	0,06	11,98	0,19	0,64	27,07	0,72	0,35	0,11	6,51	17,90	0,15	0,07	1,47		0,14	0,27	0,15		0,07
3318	1	4	2013	10	39	2	CT	2			2,32	0,03	1,90	0,04	1,90	0,07	4,84	0,25	0,09	3,49	0,05	0,07	9,03	0,16	0,74	26,82	1,09	0,89	0,55	8,63	30,38	0,62		2,70		0,27		0,52		0,21
3318	1	4	2013	10	39	24	CT	2			2,82		2,52	0,07	2,58	0,14	6,24	0,31	0,14	4,38	0,06	0,13	10,28	0,17	1,34	27,67	1,16	0,88	0,34	10,13	18,94	1,59	0,91	2,72	#	0,38		0,68		0,18
3318	1	4	2013	10	39	52	CT	2			3,64	0,04	3,98	0,10	4,24	0,08	12,10	0,47	0,09	5,90	0,13	0,06	12,65	0,16	0,64	27,13	0,60	0,39	0,16	6,29	17,31	0,19		1,59		0,14	0,16	0,15		0,07
3361	1	3	2013	9	38	22	CT	2	CT	2	3,73	0,04	3,42	0,07	3,66	0,12	9,13	0,39	0,15	6,22	0,11	0,11	11,65	0,19	0,94	26,49	0,75	0,31		8,12	19,57	0,32		2,19		0,20		0,42		0,11
3361	1	3	2013	9	38	44	CT	2	CT	2	4,58	0,04	4,47	0,06	5,02	0,19	13,21	0,46	0,12	6,99	0,19	0,09	12,01	0,16	0,75	25,34	0,56	0,25	0,10	6,39	14,07	0,75	0,32	1,56		0,15	0,10	0,27		0,08
3361	1	3	2013	9	38	72	CT	2	CT	2	3,47	0,03	3,91	0,06	4,61	0,09	13,84	0,47	0,11	7,69	0,20	0,08	13,36	0,20	0,72	23,75	0,62	0,33	0,12	6,71	14,93	0,18	0,07	1,58		0,15	0,30	0,32		0,06
3398	1	2	2013	9	38	34	CT	2																																
3398	1	2	2013	9	38	56	CT	2			4,07	0,03	3,77	0,22	3,56	0,10	9,42	0,34	0,10	5,11	0,11	0,11	10,41	0,15	1,02	29,23	0,96	0,51	0,18	8,99	14,90	0,97	0,48	1,47	#	0,21	0,29	0,27		0,11
3398	1	2	2013	9	38	84	CT	2																																
3403	1	3	2013	10	33	3	CT	2			2,64		1,69		1,47	0,04	3,46	0,21	0,06	2,82		0,06	8,69	0,18	0,64	25,77	1,12	0,94	0,45	8,70	32,59	0,87		2,92		0,34	0,23	0,47	#	0,37
3403	1	3	2013	10	33	25	CT	2			1,91	0,03	1,65	0,05	1,78	0,09	4,79	0,23	0,12	3,94	0,10	0,13	10,58	0,28	1,27	28,78	1,37	0,89	0,35	11,63	21,60	0,00	0,91	2,95	#	0,47	0,38	0,19		0,28
3403	1	3	2013	10	33	53	CT	2			9,11	0,09	8,98	0,14	8,84	0,29	22,83		0,24	10,17	0,28	0,11	12,72	0,20	0,54	14,64	0,35	0,12		1,99	4,97	0,34	0,15	0,53		0,07	0,04	0,08		
3424	1	3	2013	10	35	7	CT	2			3,30	0,06	3,23		2,72	0,10	6,84	0,36	0,14	4,85	0,08	0,12	10,29	0,20	1,00	29,11	0,91	0,61	0,24	7,88	20,54	1,43		2,21		0,23	0,25	0,44		0,24
3424	1	3	2013	10	35	29	CT	2			2,18	0,03	1,93	0,05	2,10	0,09	5,58	0,27	0,11	4,37	0,13	0,12	10,09	0,26	1,21	28,86	0,77	0,79	0,36	10,78	22,88		1,06	2,96		0,41		0,57		0,27
3424	1	3	2013	10	35	57	CT	2			4,47	0,04	4,65	0,08	4,84	0,14	11,42	0,62	0,12	5,86	0,18	0,09	9,80	0,20	0,84	24,19	0,88	0,44	0,16	5,78	15,39	1,47	0,58	2,33	#	0,29	0,29	0,60		0,13
3443	1	3	2013	10	34	7	CT	2	CT	2																														
3443	1	3	2013	10	34	29	CT	2	CT	2	2,71		2,43	0,08	2,57	0,16	6,78	0,36	0,21	5,18	0,10	0,18	12,06	0,25	1,63	29,57	1,04	0,66	0,19	9,23	15,40	1,51	0,84	2,47	#	0,37	0,45	0,37		0,24
3443	1	3	2013	10	34	57	CT	2	CT	2	4,02	0,04	4,75	0,10	5,03	0,11	15,05	0,54	0,14	6,83	0,17	0,09	13,79	0,21	0,84	26,89	0,64	0,35	0,13	5,06	11,26	0,19	0,10	1,25	#	0,17	0,22	0,37		0,09
3456	1	3	2013	10	36	7	TT	3	CT	2																														
3456	1	3	2013	10	36	29	TT	3	CT	2	2,66		2,26	0,06	2,29	0,12	[illegible]	0,27	0,14	4,51	0,07	0,14	[illegible]	0,19	1,30	27,99	0,87	0,75	[illegible]	9,14	20,44	1,53	0,84	2,91	#	0,46	0,46	0,36		0,34
3456	1	3	[illegible]	[illegible]	36	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]																														
3457	1	3	[illegible]	[illegible]	36	[illegible]	[illegible]	3	[illegible]	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5,14	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	28,48	[illegible]	[illegible]	[illegible]	[illegible]	17,92	1,64	1,04	2,82	#	0,44	0,79	0,47		0,33

ACAA2 association with FA profile

Trait	CC^2	n	CT^2	n	TT^2	n
C9:0	0.113^{c} ± 0.004	191	0.109^{c} ± 0.002	526	0.115^{d} ± 0.004	238
C11:0	0.136 ± 0.005					
C12:1 cis-9	0.152^{d} ± 0.005					
C13:0	0.089 ± 0.003					
ω6/ω3	13.67^{c} ± 0.29					

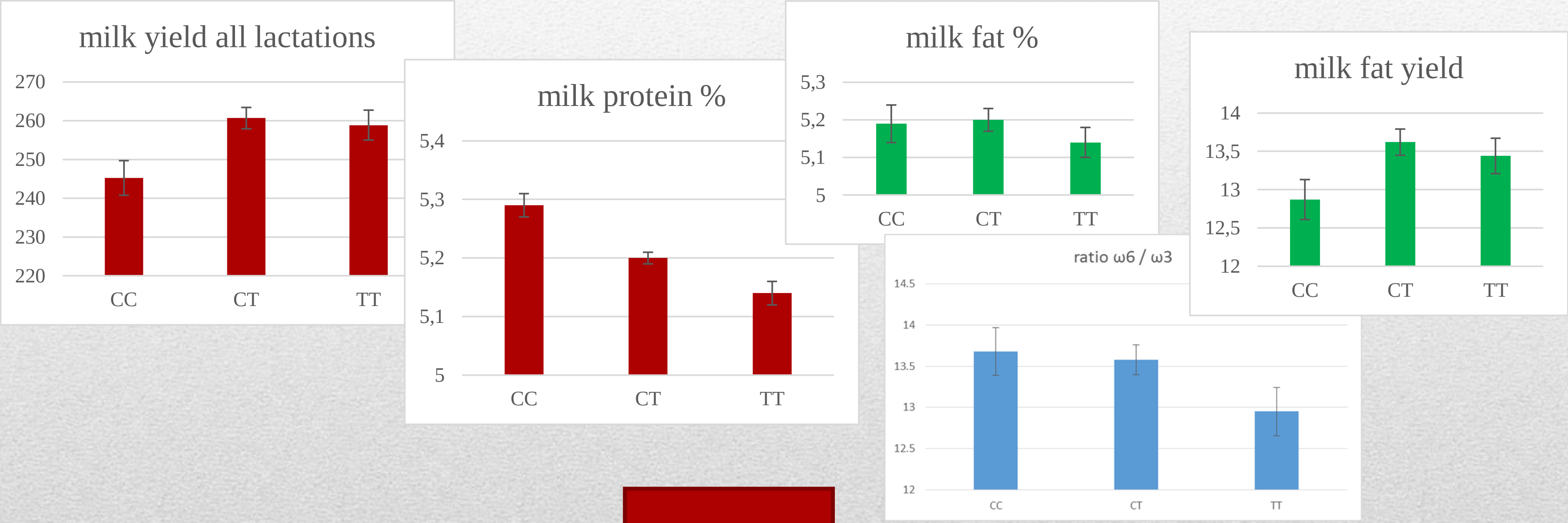


ACAA2 WAS ASSOCIATED WITH ω6/ω3 RATIO

Simoni Symeou[a], Ouranios Tzamaloukas[a], Georgios Banos[b,c], Despoina **Miltiadou[a]**
ACAA2 and *FASN* polymorphisms affect fatty acid profile of Chios sheep milk; under reviewing in Journal of Dairy Research

ACAA2 IS ASSOCIATED WITH DAIRY TRAITS

TT and CT animals produce more milk with lower protein percentage, higher fat yield
CC animals produce less milk with higher protein percentage, TT animals with higher ω6/ω3 ratio



ACAA2 3UTR SNP
is the causative mutation?

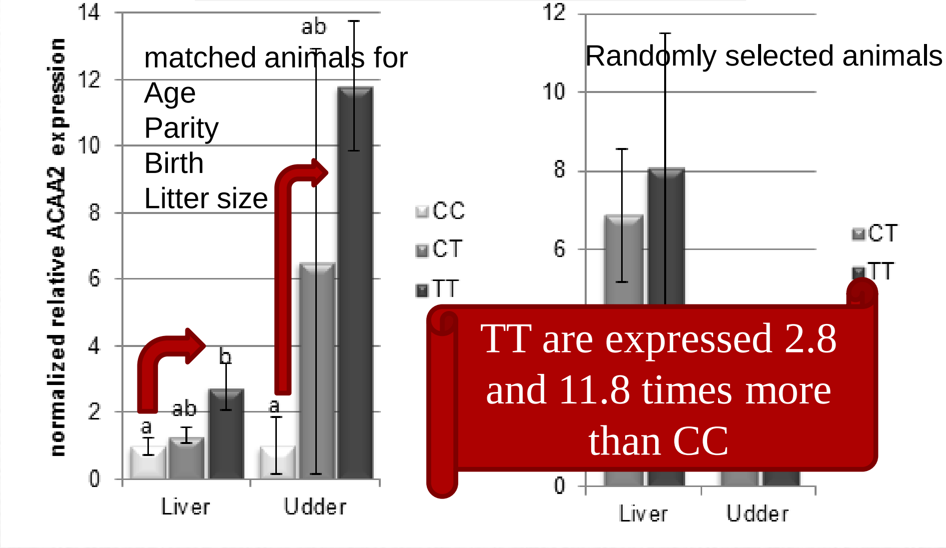
Effect
observed

ACAA2 3UTR SNP
is in linkage or LD with
the causative mutation?

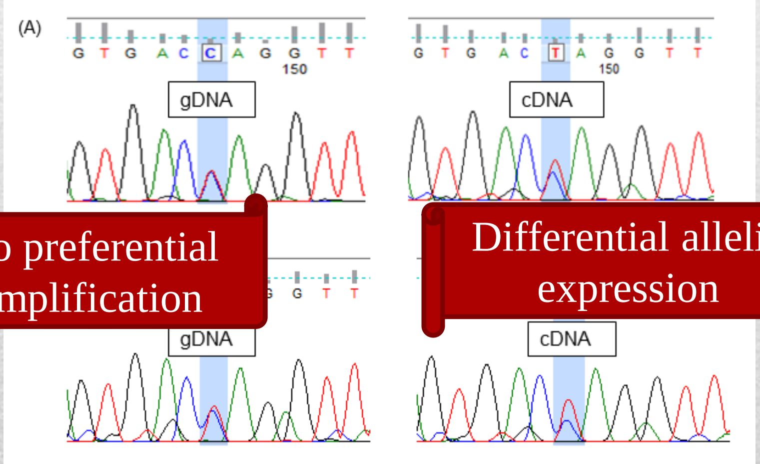
No aminoacid change, may affect the expression of the gene

__Miltiadou D.,__ Hager-Theodorides A.L., Symeou S., Constantinou C., Psifidi A., Banos G. and Tzamaloukas O.* ***Journal of Dairy Science*** *doi./10.3168/jds.2016-12326*

RNA expression analysis



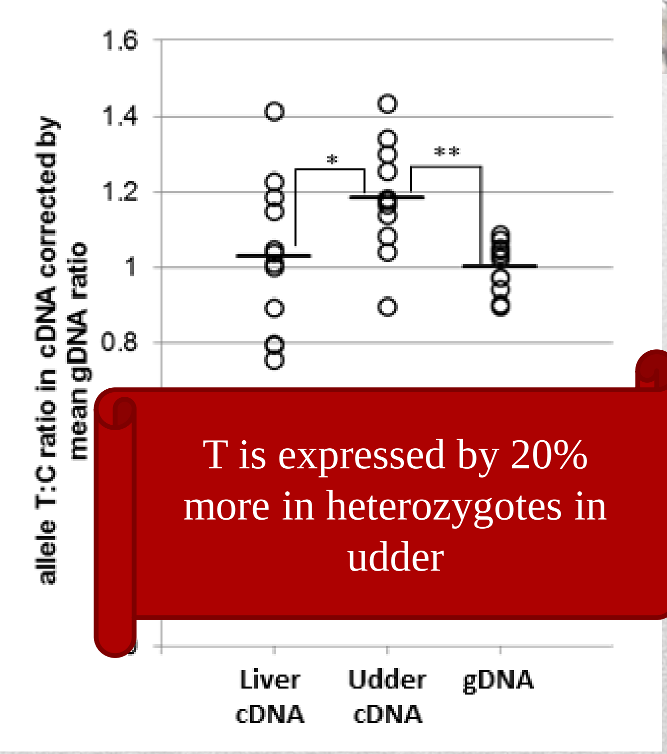
TT are expressed 2.8 and 11.8 times more than CC



No preferential amplification

Differential allelic expression

Comparison of gDNA and cDNA from heterozygous animals



T is expressed by 20% more in heterozygotes in udder

Functional ACAA2 study

Conclusions

- The variants in the 3'UTR of the *ACAA2* gene are associated
  - ➢ with milk yield
  - ➢ protein percentage
  - ➢ fat yield
  - ➢ ω6/ω3 ratio
- are differentially expressed in homozygous ewes of each allele and
- exhibit AEI within heterozygotes in a tissue specific manner

=>suggesting the existence of a cis-acting regulatory DNA mechanism.

⬇

↑mRNA expression=>likely ↑ACAA2 activity=> ↑energy and carbon substrates for lactation and FA synthesis

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Thank you for your attention !