Effects of DGAT1, FABP4, FASN, PPARGC1A, SCD1, SREBP-1 and STAT5A Gene Polymorphisms on the Fatty Acid Composition in Fleckvieh Bulls

Barton L., Bures D., Kott T., Kottova, B.

Institute of Animal Science, Prague, Czech Republic

Abstract— **The objective of this study was to confirm the presence of previously reported allelic variants in diacylglycerol acyltransferase 1 (DGAT1; g.10433_10434delinsAA), fatty acid binding protein 4 (FABP4; c.220A>G), fatty acid synthase (FASN; g.16024G>A, g.17924A>G), peroxysome proliferator-activated receptor-γ coactivaror-1α (PPARGC1A; c.1790+514G>A, c.1892+19C>T), stearoyl-coenzyme A desaturase 1 (SCD1; c.878C>T), sterol regulatory element binding protein-1 (SREBP-1; 84-bp del), and signal transducer and activator of transcription 5A (STAT5A; g.9501G>A) in Fleckvieh cattle. In addition, we evaluated the single effects of these variants on the fatty acid (FA) composition of intramuscular and subcutaneous fat in a total of 602 bulls. Except for one (AA genotype for PPARGC1A, c.1790+514G>A), all the evaluated allelic variants were present in the analysed population but significant effects on FA composition (P<0.05) were only determined for the SCD1 and both FASN polymorphisms. The SCD1 c.878C>T genotype was associated with concentrations of stearic, myristoleic and oleic acids. The FASN g.16024G>A and g.17924A>G genotypes did not explain the same part of FA variation and were mainly associated with concentrations of myristic, palmitic, myristoleic and oleic acids. It is concluded that the genetic variations in SCD1 and FASN genes contribute to the variability of FA composition in intramuscular and subcutaneous fat of Fleckvieh cattle.**

Keywords— **Beef, fatty acid, gene polymorphism.**

I. INTRODUCTION

Fatty acid (FA) composition of bovine adipose tissue is an important meat quality trait with implications for human health [1]. It was previously demonstrated that several polymorphisms in FA synthesis and metabolism related genes may be partly responsible for the between-animal variation in meat and milk fat FA composition [2, 3, 4, 5].

The objective of this study was to confirm the existence of the previously reported polymorphisms in the coding regions of diacylglycerol acyltransferase 1 (DGAT1), fatty acid binding protein 4 (FABP4), fatty acid synthase (FASN), peroxysome proliferator-activated receptor-γ coactivaror-1α (PPARGC1A), stearoyl-coenzyme A desaturase 1 (SCD1), sterol regulatory element binding protein-1 (SREBP-1), and signal transducer and activator of transcription 5A (STAT5A) in Fleckvieh bulls and to assess the independent effects of the allelic variants on the FA composition of muscle (MF) and subcutaneous fat (SF).

II. MATERIAL AND METHODS

Muscle samples of 602 Fleckvieh bulls were collected at slaughter to obtain genomic DNA. The animals were fattened under similar feeding and housing conditions in a growth performance test station for the progeny of Fleckvieh sires and slaughtered at a similar age of 530 ± 10 d.

Muscle samples were collected from *m. longissimus thoracis* (MLT) between 7th and 8th ribs whereas subcutaneous fat was taken from the brisket. Homogenised muscle samples were analysed for the neutral lipid fraction content by extraction with petroleum ether in the Soxtec Avanti 2055 apparatus (FOSS Tecator AB, Höganäs, Sweden). The FA composition of MF and SF was determined after the extraction of total lipids in accordance with [6]. Alkaline trans-methylation of FA was performed in accordance with [7]. Gas chromatography of FA methyl esters was performed using the HP 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) with a programmed 60 m DB-23 capillary column (150 to 230°C). FA were identified on the basis of retention times corresponding to standards (PUFA 1, PUFA 2, PUFA 3, 37 Component

FAME Mix; Supelco, Bellefonte, PA, USA). The proportions of muscle and subcutaneous fat FA were expressed as percentages of the total area of injected methyl esters.

Previously reported allelic variants were determined in DGAT1 (g.10433_10434delinsAA), FABP4 (c.220A>G), FASN (g.16024G>A, g.17924A>G), PPARGC1A (c.1790+514G>A, c.1892+19C>T), SCD1 (c.878C>T), SREBP-1 (84-bp del), and STAT5A (g.9501G>A). Genotyping for the polymorphisms in DGAT1, SCD1 and SREBP-1 was performed with the primers described by [4, 8, 9]. For genotyping FABP4, FASN, PPARGC1 and STAT5A, TaqMan® MGB assays were designed.

Allele and genotypic frequencies as well as the pair-wise linkage disequilibrium between the SNPs within the FASN gene were analysed using the PowerMarker v.3.25 software [10]. FA composition data were analysed with a mixed linear model and parameters were estimated using the REML method using the MIXED procedure of SAS [11]. The model included the random effects of sire and the group of contemporary animals and the fixed effect of a single-locus genotype. The differences between genotype groups within a gene were tested by Tukey's procedure.

III. RESULTS AND DISCUSSION

Genotypic and minor allele frequencies are shown in Table 1. There were 2 animals with the minor genotype KK (DGAT1), 140 II (FABP4), 28 TT (FASN g.16024G>A), 46 TT (FASN g.17924A>G), 14 TT (PPARGC1A c.1892+19C>T), 0 AA (PPARGC1A c.1790+514G>A), 141 VV (SCD1), 5 SS (SREBP-1), and 57 GG (STAT5A).

Of all the allelic variants evaluated, significant effects on FA composition ($P<0.05$) were only determined for SCD1 and both FASN polymorphisms. The FASN gene encodes the protein fatty acid synthase (FAS) which produces 16-C or shorter-chain SFA from acetyl-CoA and malonyl-CoA [12]. The effects of the FASN g.16024G>A polymorphism on the FA composition of MF and SF are given in Table 2. The T allele decreased the proportions of C14:0, C16:0 and C14:1, and increased the proportions of C18:1 and MUFA in MF. The results were generally similar for SF. In agreement with our study, corroborative genotype effects on adipose tissue FA composition were reported for Japanese Black cattle [2, 12]. No such effects were, however, observed in Holstein steers [13].

Table 1: Genotypic and minor allele frequencies (n = 602)

Gene	Polymorphism	Genotype frequencies			MAF[1]
DGAT1	g.1043_1044	AA	AK	KK	K
	delinsAA	0.89	0.11	0.00	0.06
FABP4	c.220A>G	II	IV	VV	I
		0.24	0.50	0.26	0.49
FASN	g.16024G>A	AA	AT	TT	T
		0.60	0.35	0.04	0.22
FASN	g.17924A>G	TT	TA	AA	T
		0.08	0.35	0.57	0.26
PPARGC1A	c.1892+19C>T	CC	CT	TT	T
		0.70	0.28	0.02	0.16
PPARGC1A	c.1790+514G>A	GG	GA	AA	A
		0.81	0.19	0.00	0.09
SCD1	c.878C>T	AA	AV	VV	V
		0.28	0.49	0.23	0.47
SREBP-1	84-bp Del	LL	LS	SS	S
		0.84	0.15	0.01	0.08
STAT5A	g.9501G>A	GG	GA	AA	G
		0.09	0.41	0.50	0.30

[1] MAF – minor allele frequency

The FASN g.17924A>G is the polymorphism in the TE domain of the FASN gene which plays an essential role in the determination of the product length [5]. In our study, the proportions of C14:0, C16:0, C14:1, C16:1, and SFA were lower and the proportions of C18:1 and MUFA were higher for the AA genotype in both MF and SF (Table 3). These associations were in good agreement with the results reported for total muscle lipids of Aberdeen Angus [5] and Hanwoo [14] cattle.

The SCD1 gene encodes stearoyl-CoA desaturase, a rate-limiting enzyme responsible for the conversion of SFA into MUFA. The present study confirmed our previous results obtained using fewer animals [15]. The AA genotype showed lower proportions of C18:0 and SFA and higher proportions of C14:1, C18:1 and MUFA in MF than the VV genotype. Similar associations were also demonstrated in previous reports using different cattle breeds [16, 12, 13].

Table 2: Effect of FASN polymorphism (g.16024G>A) on FA composition of muscle and subcutaneous fat (LSM ± *SE*)

	Muscle fat			Subcutaneous fat		
	AA (n = 361)	AT (n = 213)	TT (n = 28)	AA (n = 218)	AT (n = 147)	TT (n = 21)
C14:0	2.64[a] ±0.03	2.49[b] ±0.04	2.37[b] ±0.09	3.01[a] ±0.04	2.78[b] ±0.05	2.52[c] ±0.11
C16:0	26.2[a] ±0.13	25.8[b] ±0.15	25.8[ab] ±0.33	24.9 ±0.19	24.6 ±0.21	24.3 ±0.44
C18:0	18.8[a] ±0.15	19.2[b] ±0.18	18.7[ab] ±0.38	10.5 ±0.19	10.3 ±0.21	10.7 ±0.43
C14:1	0.39[a] ±0.01	0.35[b] ±0.01	0.33[ab] ±0.02	1.36[a] ±0.04	1.29[ab] ±0.04	1.15[b] ±0.09
C16:1	2.62 ±0.04	2.57 ±0.04	2.55 ±0.10	6.61[a] ±0.14	6.57[a] ±0.16	5.79[b] ±0.30
C18:1	36.7[a] ±0.19	37.3[b] ±0.23	38.5[b] ±0.53	44.5[a] ±0.26	45.4[b] ±0.29	46.4[b] ±0.60
SFA	47.7 ±0.22	47.5 ±0.22	46.9 ±0.47	38.4 ±0.32	37.7 ±0.36	37.5 ±0.75
MUFA	39.7[a] ±0.21	40.3[ab] ±0.25	41.4[b] ±0.58	52.5[a] ±0.30	53.2[b] ±0.33	53.3[ab] ±0.70
PEE[d]	35.2 ±1.17	36.1 ±1.37	40.7 ±3.12			

[a, b, c] Values in the same row with different superscripts for either muscle or subcutaneous fat differ at P<0.05.
[d] PEE - Petroleum ether extract (g/kg muscle)

Table 3: Effect of FASN polymorphism (g.17924A>G) on FA composition of muscle and subcutaneous fat (LSM ± *SE*)

	Muscle fat			Subcutaneous fat		
	TT (n = 46)	TA (n = 213)	AA (n = 343)	TT (n = 39)	TA (n = 135)	AA (n = 212)
C14:0	2.83[a] ±0.07	2.68[a] ±0.04	2.48[b] ±0.03	3.33[a] ±0.08	3.01[b] ±0.05	2.75[c] ±0.04
C16:0	26.7[a] ±0.28	26.3[a] ±0.15	25.8[b] ±0.13	25.5[a] ±0.35	25.0[a] ±0.21	24.4[b] ±0.19
C18:0	19.1 ±0.32	18.8 ±0.18	19.0 ±0.16	10.8 ±0.35	10.4 ±0.22	10.5 ±0.20
C14:1	0.43[a] ±0.02	0.38[ab] ±0.01	0.36[b] ±0.01	1.43[ab] ±0.07	1.38[a] ±0.04	1.27[b] ±0.04
C16:1	2.67[ab] ±0.08	2.66[a] ±0.04	2.56[b] ±0.04	6.65 ±0.25	6.69 ±0.16	6.45 ±0.14
C18:1	35.8[a] ±0.43	36.5[a] ±0.23	37.5[b] ±0.20	43.3[a] ±0.47	44.4[a] ±0.29	45.6[b] ±0.25
SFA	48.6[a] ±0.39	47.9[a] ±0.21	47.3[b] ±0.19	39.7[a] ±0.60	38.4[ab] ±0.36	37.6[b] ±0.32
MUFA	38.9[a] ±0.48	39.5[a] ±0.25	40.4[b] ±0.22	51.4[a] ±0.55	52.5[a] ±0.34	53.3[b] ±0.30
PEE[d]	33.4 ±2.61	35.8 ±1.38	36.1 ±1.20			

[a, b, c, d] See Table 2 for explanation.

Table 4: Effect of SCD1 polymorphism (c.878C>T) on FA composition of muscle and subcutaneous fat (LSM ± *SE*)

	Muscle fat			Subcutaneous fat		
	AA (n = 171)	AV (n = 290)	VV (n = 141)	AA (n = 103)	AV (n = 193)	VV (n = 90)
C14:0	2.60 ±0.04	2.54 ±0.04	2.62 ±0.04	2.89[ab] ±0.06	2.85[a] ±0.05	3.01[b] ±0.06
C16:0	26.2 ±0.16	25.8 ±0.14	26.2 ±0.17	24.9 ±0.24	24.6 ±0.20	24.7 ±0.25
C18:0	18.6[a] ±0.19	19.0[ab] ±0.16	19.3[b] ±0.20	10.2[a] ±0.23	10.4[ab] ±0.20	10.9[b] ±0.24
C14:1	0.45[a] ±0.01	0.36[b] ±0.01	0.31[c] ±0.01	1.54[a] ±0.04	1.30[b] ±0.03	1.10[c] ±0.04
C16:1	2.58 ±0.05	2.59 ±0.04	2.65 ±0.05	6.45 ±0.18	6.62 ±0.15	6.53 ±0.18
C18:1	37.2[a] ±0.25	37.2[a] ±0.21	36.4[b] ±0.27	44.9 ±0.33	45.1 ±0.28	44.6 ±0.35
SFA	47.5[a] ±0.24	47.4[a] ±0.20	48.2[b] ±0.25	38.0 ±0.40	37.9 ±0.33	38.6 ±0.42
MUFA	40.3[a] ±0.28	40.2[a] ±0.23	39.4[b] ±0.29	52.9 ±0.37	53.0 ±0.30	52.3 ±0.39
PEE[d]	40.0[a] ±1.58	33.9[b] ±1.25	34.4[b] ±1.48			

[a, b, c, d] See Table 2 for explanation.

The polymorphisms in DGAT1 (g.1043_1044 delinsAA) and PPARGC1A (c.1892+19C>T; c.1790+514G>A) were shown to affect the FA composition of milk [4, 17]. Also, significant differences between the FABP4 genotypes (c.220A>G) were found in C16:0 [13] and C16:1 [3] obtained from bovine muscle and subcutaneous fat. No such relationships were, however, observed for bovine adipose tissue in our study.

IV. CONCLUSIONS

It is concluded that the genetic variations in SCD1 (c.878C>T) and FASN (g.16024G>A and g.17924A>G) genes contribute to the variability of FA composition in MF and SF of Fleckvieh cattle. The observed associations indicate that these mutations may have an influence on gene function.

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