Preliminary Association Studies Between EBV And SNPs In 5 Candidate Genes For Milk Fat In Goats.

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Introduction

Fat yield and content play a major role in the technological and nutritional quality of goat milk, influencing nutritional, aromatic and technological characteristics, like firmness, color and flavor, of milk and milk-derived products (Delacroix-Buchet and Lamberet 2000). These traits are relevant factors for the market value of dairy goat products and make milk fat a considerable component of the economic efficiency of goat production. Moreover, fatty acid composition of milk is of great interest, considering the implications for human health (Garcìa-Fernandez et al. 2009).

This work aims to present some preliminary results of the association studies between a panel of SNPs identified in candidate genes for milk fat and estimated breeding values for fat yield (kg) and fat content (%), calculated by the National Breeders Association (ASSONAPA) on a sample of 80 Alpine and Saanen bucks reared in Italy.

The five candidate genes investigated are well known for their role. Acetyl-coenzyme A carboxylase alpha (ACACA) is part of a multifunctional complex enzyme system which catalyzes the rate-limiting step in fatty acid synthesis. Badaoui et al. (2007a) observed in ACACA gene a polymorphism associated with fat yield, lactose content and somatic cell count in 4 Spanish goat breeds. Diacilglycerol acyl transferase 1 (DGAT1) is a key enzyme in triacylglycerol synthesis in the mammary gland, well known in cattle for its effects on fat and protein yield (Grisart et al. 2004), whereas in goat Angiolillo et al. (2007) identified only a SNP. Fatty acid synthase (FASN) encodes a multifunctional protein catalyzing the synthesis of palmitate into long-chain saturated fatty acids. In cattle SNP on FASN gene showed a significant association with milk fat yield and content (Roy et al. 2006). Lipoprotein lipase (LPL) encodes homonymous lipase with the dual functions of triglyceride hydrolase and ligand/binding factor for receptor-mediated lipoprotein uptake. In this gene Badaoui et al. (2007) observed one SNP associated with fat content in 4 Spanish goat breeds. Stearoyl-CoA desaturase (SCD) is the key enzyme involved in the endogenous synthesis of conjugated linoleic acid (CLA) in ruminants. It is well studied in cattle whereas in goat only some SNPs are known (Badaoui et al. 2007b; Moioli et al. 2007).

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Material and methods

A total of 80 animals belonging to two breeds: Alpine (n=37) and Saanen (n=43) were studied. The biological samples were provided by LGS, the National Association of Breed and Species Laboratory. Genomic DNA was extracted from hair bulb using a commercial kit (Promega) and following the manufacturer’s instructions. SNP discovery was carried out by sequencing, aligning and comparing the PCR products of 12 animals belonging to different breeds, including the studied ones. The panel of 80 animals was then genotyped for each of the identified SNPs by an outsourcing service (http://kbioscience.co.uk).
Using the Power Marker v.3.25 software (http://statgen.ncsu.edu/powermarker), we calculated the main genetic parameters for the studied SNPs. The association analysis was performed between SNPs and Estimated Breeding Values for fat yield (kg) and fat content (%), estimated by the National Breeder Association (ASSONAPA) on Alpine and Saanen bucks. Allelic substitution effect on fat yield and content was estimated as a regression coefficient as in Sherman et al. 2008, using JMP v.3.2.2 software (Sas Inst., 1989).

Results and discussion

We used 18 SNPs for the association analysis. The genetic parameters obtained on the 80 animals for the SNP analyzed were reported in table 1.

Table 1: Main genetic parameters on SNP markers in the 80 bucks studied

SNP	MAF	Genotype obs. n.	H. exp.	H. obs.	PIC	Fst	Reference
ACACAex14_CT	0,83	2	0,29	0,35	0,25	0,0099	This work
ACACAex45_CT	0,75	3	0,38	0,28	0,3	-0,018	Badaoui, 2007
ACACAex46_1CT	0,96	2	0,08	0	0,08	0,0594	This work
ACACAex46_2CT	0,95	2	0,09	0,09	0,08	0,0765	This work
DGAT1int12_AG	0,93	3	0,12	0,04	0,12	0,1084	This work
DGAT1int14_insC	0,91	3	0,16	0,1	0,15	-0,012	This work
DGAT1int16_C703T	0,91	3	0,17	0,16	0,15	0,1073	Angiolillo , 2007
DGAT1int7_CT	0,99	2	0,03	0,03	0,03	-0,013	This work
FASNex18_CT	0,79	3	0,33	0,34	0,28	0,032	This work
FASNex34_CT	0,91	3	0,16	0,15	0,15	0,1094	This work
FASNint16_CT	0,69	3	0,43	0,48	0,34	0,0212	This work
FASNint3_1AG	0,56	3	0,49	0,48	0,37	0,0099	This work
FASNint3_2GC	0,85	3	0,26	0,27	0,22	0,0351	This work
LPLex1_C50G	0,85	3	0,26	0,22	0,23	-0,01	Badaoui, 2007
LPLint7_CT	0,86	3	0,24	0,19	0,21	0,0332	This work
SCD3UTR_delTGT	0,59	3	0,48	0,42	0,37	-0,012	This work
SCDex2_AG	0,65	3	0,46	0,4	0,35	-0,006	This work
SCDex3_AG	0,61	3	0,48	0,47	0,36	-0,004	This work
SCDex5_CT	0,63	3	0,47	0,41	0,36	-0,018	Yahyaoui, 2003
SCDex5_GT	0,78	3	0,35	0,26	0,29	-0,009	Yahyaoui, 2003

We identified 13 SNPs in the above reported genes (ACACA n.3; DGAT1 n.2; FASN n.5; LPL n.1; SCD n.2), whereas 5 SNP were previously reported by other authors in ACACA, DGAT1, LPL and SCD genes (Angiolillo et al. 2007; Badaoui et al. 2007ab; Yahyaoui et al. 2003). 11 SNPs are in exons, 8 in introns and 1 in 3'UTR region. SNPs in the exon 3 and 5 of SCD gene are non synonymous. The details on these SNP will be reported in a paper in progress. 3SNP show rare allele (MAF $\geq$ to 0.95) and 2 out of the 3 possible genotypes. Also the SNP on exon 45 of the ACACA gene shows only 2 genotypes.

The estimates of the allele substitution effect are reported in table 2. On fat yield 4 SNPs show a significant allelic substitution effect whereas on fat content only 2 SNPs show a significant association. The polymorphism observed in exon 14 of ACACA gene shows the positive substitution effect for the minor allele only in Saanen bucks. Whereas the SNP in exon 5 of the ACACA gene is the only one showing a significant effect in both the studied breeds for fat content with a negative substitution effect for the minor allele. Moreover, in the Saanen breed this SNP results also associated to fat content (P=0.037) and the negative substitution effect is due to the minor allele (0.14). These observations agree with previous observations. The association of this synonymous SNP with fat content (%), lactose content (%) and somatic cell count (SCC) has been reported also for four Spanish goat breeds (Badoui et al., 2007).

The SNP observed in the intron 16 of FASN gene, showing that the highest significant effect (p=0.006) is a positive substitution effect for the major allele in Alpine breed.

The last SNP having a significant effect on fat yield is located in intron 7of LPL gene and the major allele observed in Alpine breed has a strong negative substitution effect.

On fat content (%) beyond the polymorphism in exon 45 also one in exon 46 of the ACACA gene shows a significant effect in Saanen breed, but the minor allele shows a positive substitution effect.

In the last column of table 2 is reported the substitution effect in SD of relative trait EBVs.

The SNP on exon 46 of ACACA gene has the most relevant effect, followed by the SNP on LPL gene but with a negative connotation tied to the major allele.

Table 2: Estimates of the allele substitution (alpha) effect and breed frequencies.

EBV	SNP	Allele	Frequency	Alpha	P value	Breed	Alpha DS
Fat yield (kg)	ACACAex14_CT	T	0.14	0.936	**0.052**	SAA	0.69
	ACACAex45_CT	T	0.24	-1.145	**0.015**	ALP	-0.54
	ACACAex45_CT	T	0.26	-0.762	**0.041**	SAA	-0.56
	FASNint16_CT	T	0.60	0.184	**0.006**	ALP	0.09
	LPLint7_CT	T	0.80	-1.508	**0.021**	ALP	-0.71
Fat content (%)	ACACAex45_CT	T	0.14	-0.03	**0.037**	SAA	-0.29
	ACACAex46_2CT	T	0.09	0.10	**0.013**	SAA	1.03

Conclusion

In the present study a significant effect of 5 SNPs on estimated breeding values far fat yield and content was found in 3 out of 5 candidate genes.

The major allele of SNP in exon 45 of the ACACA gene is associated to higher EBV for fat yield in both breeds and to higher EBV for fat content in Saanen. Also, the major allele of the SNP found in FASN gene seems associated to higher EBV for fat yield. On the contrary, the major allele of the other 3 SNPs observed in ACACA and LPL genes seems associated to lower EBV values. The significant relationships between the polymorphisms recorded in the five studied candidate genes deserves to be carefully considered, given the lack of knowledge in goat and the economic importance of milk fat yield and content, for their relevant contribution to cheese yield and firmness as well as to cheese flavor and colour.

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