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Characterization of the Casein Gene Complex in West African Goats and Description of a New α_{s1}-Casein Polymorphism

A. Caroli,*[1] F. Chiatti,† S. Chessa,† D. Rignanese,† E. M. Ibeagha-Awemu,‡§ and G. Erhardt§
*Dipartimento di Scienze Biomediche e Biotecnologie, Università degli Studi di Brescia, Viale Europa 11, 25123 Brescia, Italy
†Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università degli Studi di Milano, Via Trentacoste 2, 20134 Milano, Italy
‡Department of Animal Science, McGill University, Ste-Anne-De-Bellevue, Quebec, H9X 3V9, Canada
§Institut für Tierzucht und Haustiergenetik, Justus-Liebig Universität, Ludwigstr. 21 b, 35390 Gießen, Germany

ABSTRACT

The analysis of casein polymorphisms was carried out in West Africa goat populations: Red Sokoto (n = 57), West African Dwarf Nigeria (n = 27), West African Dwarf Cameroon (n = 39), and Borno (n = 37). The 4 casein genes α_{s1} (*CSN1S1*), β (*CSN2*), α_{s2} (*CSN1S2*), and κ (*CSN3*) were typed at the DNA level. No null alleles were found in any of the genes analyzed. A PCR single-strand conformation polymorphism method was implemented for the identification of *CSN1S1*F* allele simultaneously with *A/0$_1$, B/E, N* and the new allele. The allele differed from *CSN1S1*B* by a synonymous transversion TCG→TCT in the codon corresponding to Ser_{66} of the mature protein. The new allele, named *CSN1S1*B′,* occurred at a high frequency in all the populations, ranging from 0.295 (West African Dwarf Cameroon) to 0.405 (Borno). A greater frequency was found for alleles associated with high α_{s1}-casein quantity, as has already been observed in the goat populations from the Mediterranean area. The intermediate *E* allele occurred only in the Red Sokoto and at a low frequency. The faint *F* allele occurred in 3 populations at frequencies lower than 0.03. Linkage disequilibrium occurred in all the populations, with highly significant differences in Borno, Red Sokoto, and West Africa Dwarf Nigeria, and significant differences in West Africa Dwarf Cameroon. Only 10 haplotypes showed frequencies ≥0.05 in at least 1 of the 4 populations considered, and the overall frequency was >0.1 only for 4 haplotypes: *BAAB, B′ACA, ACAB,* and *BACA* (in the order *CSN1S1-CSN2-CSN1S2-CSN3*). Haplotype *BAAB,* postulated as an ancestral haplotype in previous studies, was the most common haplotype in all breeds except Borno, where *B′ACA* was predominant. The results obtained are of considerable significance given that very little information exists on the subject for African goats. The high frequency of strong alleles in the calcium-sensitive caseins as well as the high linkage disequilibrium found among the casein genes in the African breeds analyzed may suggest that specific casein haplotypes have already been selected due to their advantages for nutrition. Haplotypes providing greater protein and casein content would increase the energy content of milk, thus resulting in more favorable growth and survival of young goats and humans consuming the milk.

Key words: casein complex, West Africa, goat, α_{s1}-casein

INTRODUCTION

Because the CN genes are tightly linked on the same chromosome (Ferretti et al., 1990; Threadgill and Womack, 1990), they are usually inherited as a gene complex, or haplotype, from parents to progeny. The entire CN gene complex spans about 250 kb on chromosome 6 in cattle, sheep, and goats (Hayes et al., 1993; Popescu et al., 1996).

Within the goat CN cluster, high polymorphism has been found at the 4 genes *CSN1S1, CSN2, CSN1S2,* and *CSN3* coding respectively for the proteins α_{s1}-CN, β-CN, α_{s2}-CN, and κ-CN. Several alleles of the 3 calcium-sensitive CN (α_{s1}-CN, β-CN, and α_{s2}-CN) are associated with a null or reduced expression of the specific protein.

Strong alleles (*A, B$_1$, B$_2$, B$_3$, B$_4$, C, H, L,* and *M*), intermediate alleles (*E* and *I*), weak alleles (*F* and *G*), and null alleles (*0$_1$, 0$_2$,* and *N*) were described in α_{s1}-CN (reviewed in Grosclaude and Martin, 1997; Rando et al., 2000; Ramunno et al., 2005).

At *CSN2,* 3 variants were associated with a normal β-CN content: *A, B* (Mahé and Grosclaude, 1993), and *C* (Neveu et al., 2002), and 2 null *CSN2* alleles were identified (Ramunno et al., 1995; Persuy et al., 1999), and named respectively as *CSN2*0′* and *CSN2*0* by Neveu et al. (2002). Two *CSN2* alleles have recently

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[1]Corresponding author: caroli@med.unibs.it

been identified: *CSN2*A1*, which was defined as a synonymous mutation of *CSN2*A* (Cosenza et al., 2005), and *CSN2*E,* which is characterized by a transversion TCT→TAT responsible for the AA substitution $\underline{\text{Ser}}_{166}\rightarrow\overline{\text{Tyr}}_{166}$ in the mature protein (Caroli et al., 2006). Data about the expression of *CSN2*A1* and *CSN2*E* are not yet available.

The *A, B* (Boulanger et al., 1984), *C* (Bouniol et al., 1994), *E* (Lagonigro et al., 2001), and *F* (Ramunno et al., 2001a) *CSN1S2* alleles are associated with a normal α_{s2}-CN synthesis level, whereas *D* and *0* are associated with lower and null synthesis levels, respectively (Ramunno et al., 2001a,b).

Of the 16 *CSN3* alleles identified and characterized in recent years (Caroli et al., 2001; Yahyaoui et al., 2001; Angiolillo et al., 2002; Yahyaoui et al., 2003; Jann et al., 2004; Prinzenberg et al., 2005), 13 are protein variants (named in alphabetical order from *A* to *M*) and 3 (*B′, B″, C′*) show silent mutations; their occurrence is detectable at the DNA level only.

Great differences have been found among goat breeds and populations in the polymorphism distribution within CN genes (Grosclaude et al., 1994; Caroli et al., 2001) and haplotypes (Sacchi et al., 2005; Caroli et al., 2006). The haplotype coding for *CSN1S1*B, CSN2*A, CSN1S2*A,* and *CSN3*B* protein variants was postulated as ancestral among the haplotypes considered by Caroli et al. (2006).

European goat breeds have been thoroughly investigated, but data are missing regarding the CN gene and haplotype variation in goats from African countries. Such information could be of great interest, not only for utilization in animal breeding, but also from a phylogenetic point of view. This paper aimed to analyze the variability of the goat CN complex in 4 West Africa goat populations. A new *CSN1S1* variant was also identified and characterized.

MATERIALS AND METHODS

Samples and Breeds

Blood samples were collected from the following West Africa goat populations: Red Sokoto (n = 57), West African Dwarf Nigeria (n = 27), West African Dwarf Cameroon (n = 39), and Borno (n = 37).

Red Sokoto Goat. The Red Sokoto goat, also known as Kano Brown or Maradi, is the most widely distributed goat breed in Nigeria. Spread over the northern two-thirds of the country, it accounts for about 60% of the Nigerian goat population, estimated at about 28 million animals (FAOSTAT, 2005). This breed is also found in southern Niger and northern Cameroon. It is a relatively small goat with an adult height at the withers of about 64 cm, BW of about 27 kg, a typical dark red or brown coat color, short and horizontal ears, and horns in both sexes (FDLPCS, 1992). Its main products include meat, milk, and hides. The skin is one of the world's most valuable (Devendra and McLeroy, 1982; FDLPCS, 1992). With an average milk yield per lactation of 46 kg (FDLPCS, 1992) and litter size at birth of 1.8 kids (Awemu et al., 1999), the Red Sokoto goat is one of the most prolific breeds of goats in the West African region. The milk of the Red Sokoto goat has high percentages of fat (5.8%), total solids (15.37%), and ash (0.77%; Malau-Aduli and Anlade, 2002).

West African Dwarf Goat. Other names for the West African Dwarf (**WAD**) goat include African Dwarf, African Pygmy, Djallonke, Forest Goat, Fouta Djallon, Grassland Dwarf, Chèvre Naine de Savanes, Guinean, Guinean Dwarf, Ghana Dwarf, Congo Dwarf, Pygmy, Tibetana, Cameroon Dwarf, Chèvre de Casamance, Diougry, Chèvre Naine de l'Est, Kosi, and Nigerian Dwarf. The WAD goat is an achondroplastic dwarf animal that is widely distributed along the West African coastline and Central Africa. It constitutes about 50% of all goat breeds in Cameroon and is the second largest goat population in Nigeria (FDLPCS, 1992; Messine et al., 1995). The major characteristics include a height of 30 to 50 cm, male adult weight of 20 to 25 kg, multicolored coat, and horns in both sexes. Even though the main use of the breed is meat, recent investigations have shown that it has the same milk-producing ability as the Red Sokoto goat (Bemji, 2003). It is considered to be an up-and-coming dairy breed due to its promising milk production characteristics such as high butterfat and protein percentage (Wetherbee, 2002; Ruminations, 2007). The WAD goat is also reputed to be trypanotolerant (Agyemang, 2005). In this work, the WAD goat was sampled both in Nigeria and in Cameroon. We decided to take into account the different geographic origin of the WAD animals to highlight eventual differences between the 2 populations.

Borno. The Borno goat is found exclusively in Bornu and surrounding states in Nigeria. Another name is Bornu White goat, as it is a predominantly white animal, thought to have originated from the Sahel (also known as Desert or West African long-legged goat) or the Nigerian goat (FAO, 2006) breeds. It is intermediate in size between the Red Sokoto goat and the Sahel goat. Horns are present in both males and females. Its purposes include meat, milk, and a source of cultural savings. It is less well studied than the Red Sokoto and WAD breeds.

Genotyping

As described by Caroli et al. (2006), DNA was extracted from blood samples by standard methods, and

typed. In addition, *CSN1S2* was typed by PCR-RFLP according to Ramunno et al. (1999) to identify the *A*, *B*, and *C* alleles at the DNA level. Moreover, *CSN2* was typed by PCR-RFLP (Cosenza et al., 2005) to check the occurrence of the *A1* mutation.

A PCR-single strand conformation polymorphism (**PCR-SSCP**) method was implemented as an alternative to the PCR-RFLP method described for the identification of *CSN1S1*F* allele (Ramunno et al., 2000). A 212- to 223-bp *CSN1S1* fragment containing exon 9 was amplified by PCR performed in a 25-μL reaction mixture containing 2 μL of DNA solution (100 to 150 ng), 10 pmol of each primer, and 1× PCR Master Mix (Fermentas, Vilnius, Lithuania). Primers were 5′-TTC TAA AAG TCT CAG AGG CAG-3′ and 5′-GGG TTG ATA GCC TTG TAT GT-3′. The following amplification conditions were used: an initial denaturation step of 94°C for 5 min was followed by 35 cycles of 94°C for 40 s, 56°C for 40 s, and 72°C for 90 min, concluding with a final extension step of 72°C for 7 min using a PTC-200 DNA Engine thermal cycler (MJ Research Inc., Waltham, MA).

For SSCP, 6 μL of PCR product was added to 8 μL of denaturing solution (0.05% xylene-cyanol, 0.05% bromophenol blue, 0.02 *M* EDTA in deionized formamide). After heat denaturation of 95°C for 8 min, the samples were immediately chilled on ice and then run overnight (17 h) on 12.5% acrylamide:bisacrylamide gels (37.5:1) with 1% glycerol in 0.5× Tris-borate-EDTA buffer (0.54% Tris, 0.27% boric acid, 0.037% EDTA) at 240 V and 5°C (Penguin Dual Gel Water-Cooled Electrophoresis System, OWL Scientific Inc., Woburn, MA). Bands were visualized by silver staining (Bassam et al., 1991).

Reference samples carrying different *CSN1S1* alleles (*CSN1S1*A, CSN1S1*B, CSN1S1*F, CSN1S1*E, CSN1S1*0$_1$, CSN1S1*N*) were used to validate the method. Moreover, 40 samples of the present work analyzed by the SSCP method described here were randomly chosen and typed also by PCR-RFLP (Ramunno et al., 2000) as a further validation test. Allele-specific PCR analyses were necessary to distinguish *CSN1S1*B* from *CSN1S1*E* (Jànsa Pérez et al., 1994) and *CSN1S1*A* from *CSN1S1*0$_1$* (Cosenza et al., 2003), both after PCR-SSCP and PCR-RFLP genotyping of *CSN1S1*F*.

The DNA samples showing previously uncharacterized patterns on SSCP gels during *CSN1S1* typing were randomly selected for sequencing. Four samples were sequenced, 1 homozygous and 3 heterozygous. Primers used for sequencing were the same used for the PCR-SSCP technique. The PCR products were sequenced by PRIMM Srl (Milan, Italy). The nucleotide sequences and the deduced AA sequences were analyzed by Bioedit software (Hall, 1999).

Statistical Analyses

The GENEPOP program (Raymond and Rousset, 1995) was used for the evaluation of allele frequencies and deviations from Hardy-Weinberg equilibrium. The CN haplotype frequencies were estimated by the EH program (Xie and Ott, 1993). For EH computation, alleles with frequencies lower than 0.05 were ignored.

RESULTS AND DISCUSSION

A New CSN1S1 Allele

The PCR-SSCP developed for the analysis of *CSN1S1* allowed the simultaneous identification of the alleles *CSN1S1*F, CSN1S1*A/0$_1$, CSN1S1*B/E,* and *CSN1S1*N,* which can also be discriminated by PCR-RFLP (Ramunno et al., 2000), as well as identification of an unknown mutation involving several samples, which was defined as *CSN1S1*B* by the standard DNA methods (Jànsa Pérez et al., 1994; Ramunno et al., 2000; Figure 1). The new allele was named *CSN1S1*B′*. It was characterized by a synonymous transversion TC<u>G</u>→TC<u>T</u> in the Ser$_{66}$ of the mature protein. As to the possible origin of this mutation in the CN complex evolution, the alignment among the *CSN1S1* sequences of different species (bovine: GenBank accession number X59856; ovine: NM_001009795, X03237; caprine: AJ504710, AJ504711, AJ504712) suggests that *CSN1S1*B′* arises from the goat ancestral *CSN1S1*B* allele. In fact, guanine occurs instead of thymine in the corresponding nucleotide position in the sequences of all these species.

Allele Variability

Table 1 shows the allele frequencies at each CN gene. Hardy-Weinberg equilibrium occurred in all loci of the 4 populations, except for a deviation (P = 0.053) at *CSN1S1* in Borno goat where an excess of homozygous animals was found (18 observed vs. 12.4 expected).

No null alleles occurred in any of the calcium-sensitive genes analyzed, whereas strong alleles were found at very high frequencies in all of them. This is a useful indication for selection programs to improve milk traits, mainly focusing on composition properties and direct consumption quality.

At *CSN1S1,* a greater frequency was found for alleles associated with high α_{s1}-CN quantity, as has already been observed in the goat populations from the Mediterranean area (Grosclaude and Martin, 1997; Tadlaoui Ouafi et al., 2002; Sacchi et al., 2005). The intermediate *E* allele occurred only in the Red Sokoto and at a low frequency. The faint *F* allele occurred in 3 populations, at frequencies lower than 0.03.

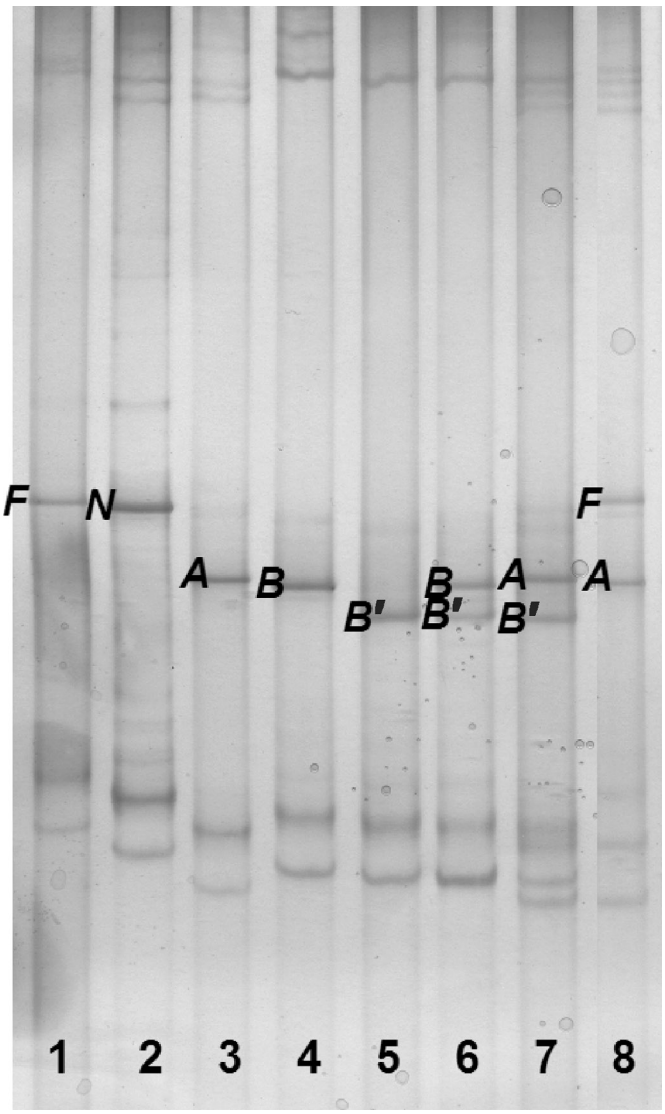

Figure 1. Analysis by PCR-single strand conformational polymorphism of goat *CSN1S1.* The discriminating band of each allele is indicated. In ascending migration mobility: *F = CSN1S1*F; N = CSN1S1*N; A = CSN1S1*A + CSN1S1*0$_1$; B = CSN1S1*B + CSN1S1*E; B′ = CSN1S1*B′.* The genotypes of the 8 samples are 1) *FF,* 2) *NN,* 3) *AA,* 4) *BB,* 5) *B′B′,* 6) *BB′,* 7) *AB′,* and 8) *AF.* The discrimination of *A* and *B* alleles respectively from *0* and *E* was performed successively by allele-specific PCR (Jànsa Pérez et al., 1994; Cosenza et al., 2003).

The most common *CSN1S*1 allele was *B*. The new *CSN1S1*B′* allele also occurred at a high frequency in all the populations. The *A* allele was the third most common allele in all populations.

As far as *CSN2* is concerned, the *A* allele was by far the most common, with a frequency exceeding 0.7 in all the populations. Only *CSN2*C* was found as an alternative to *CSN2*A,* except for in a Red Sokoto goat carrying an *A1* allele in an *A1C* genotype. This sample was defined as *CC* by PCR-SCCP analysis (Chessa et al., 2005a). Most probably, the synonymous mutation found by Cosenza et al. (2005) involves both *CSN2*A* and *CSN2*C.* In this case, we should name the allele as *CSN2*C1* instead of *CSN2*A1.* Due to the low frequency of the synonymous mutation in the populations analyzed, the nomenclature proposed by Cosenza et al. (2005) has been maintained, waiting for further investigations in different breeds.

The predominant *CSN1S2* variant was *A*, followed by *C*. The *B* and *F* alleles were absent or rare in these breeds.

Also, *CSN3* may be considered biallelic for *A* and *B*; the *M* allele was observed only in the Red Sokoto, and at a low frequency. The *A* and *B* frequencies were similar, with higher values for *CSN3*B* except in Borno. In European breeds, *CSN3*B* is usually the highly prevalent allele (Yahyaoui et al., 2003; Prinzenberg et al., 2005; Sacchi et al., 2005; Caroli et al., 2006).

A clear predominance of the proposed goat ancestral alleles *CSN1S1*B* (Grosclaude et al., 1994), *CSN2*A* (Chessa et al., 2005a), and *CSN1S2*A* (Sacchi et al., 2005) is noticeable, whereas mutated variants at the calcium-sensitive CN are usually most common in the European goat breeds; that is, *CSN1S1*F, CSN2*C,* and *CSN1S2*F* (Caroli et al., 2006).

Significant differences in allele frequencies between the 2 WAD populations were found for all the alleles except for the novel *CSN1S1*B′* variant. This discrepancy linked to the geographic origin of the samples was also confirmed at the haplotype level (Table 2) and may be the consequence of genetic drift.

Haplotype Distribution

Haplotype frequencies at the *CSN1S1-CSN2-CSN1S2-CSN3* cluster are reported in Table 2. A total of 24 possible haplotypes resulted from the combination of 3-2-2-2 alleles considered. The haplotype frequencies expected under the independence hypothesis (**IF**) were strongly different from the haplotype frequencies estimated by the EH program taking association into account (**AF**).

Only 10 haplotypes showed AF values ≥0.05 in at least 1 of the 4 populations considered, and the overall AF frequency was >0.1 only in 4 of them: *BAAB, B′ACA, ACAB,* and *BACA* (in the order: *CSN1S1-CSN2-CSN1S2-CSN3*). A high frequency of the *BAAB* haplotype, postulated as ancestral in previous studies (Sacchi et al., 2005; Caroli et al., 2006), was observed in all the populations. It was the most common haplotype in all breeds except Borno, where *B′ACA* was predominant (AF = 0.25), followed by *ACAB* (0.159), *BAAA* (0.157), and *BAAB* (0.123).

Table 1. Allele frequencies at the casein loci in Borno (BG), Red Sokoto (RS), West African Dwarf Cameroon (WADC), and West African Dwarf Nigeria (WADN) breeds

		Breed			
Locus	Allele	BG (n = 37)	RS (n = 57)	WADC (n = 39)	WADN (n = 27)
CSN1S1	*A*	0.189	0.175	0.077	0.259
	B	0.378	0.482	0.615	0.444
	B′	0.405	0.316	0.295	0.296
	E	—[1]	0.018	—	—
	F	0.027	0.009	0.013	—
CSN2	*A*	0.703	0.746	0.833	0.722
	A1	—	0.009	—	—
	C	0.297	0.245	0.167	0.278
CSN1S2	*A*	0.55	0.596	0.590	0.759
	B	—	0.026	—	—
	C	0.446	0.368	0.397	0.241
	F	—	0.009	0.013	—
CSN3	*A*	0.514	0.412	0.311	0.407
	B	0.486	0.570	0.689	0.593
	M	—	0.018	—	—

[1]— indicates allele frequency = 0.

Table 2. Haplotype frequencies at the casein loci in Borno (BG), Red Sokoto (RS), West African Dwarf Cameroon (WADC), and West African Dwarf Nigeria (WADN) breeds[1]

Casein loci				Breed									
				BG (n = 35)		RS (n = 47)		WADC (n = 36)		WADN (n = 27)		Overall[2]	
CSN1S1	CSN2	CSN1S2	CSN3	IF[3]	AF[4]	IF	AF	IF	AF	IF	AF	IF	AF
A	*A*	*A*	*A*	0.042	—[5]	0.034	—	0.013	—	0.058	—	0.026	—
A	*A*	*A*	*B*	0.035	—	0.044	—	0.028	—	0.084	—	0.038	—
A	*A*	*C*	*A*	0.035	—	0.028	—	0.009	0.014	0.018	0.019	0.015	0.007
A	*A*	*C*	*B*	0.030	—	0.037	—	0.020	—	0.027	—	0.021	—
A	*C*	*A*	*A*	0.017	—	0.012	0.027	0.002	0.014	0.022	0.039	0.008	0.019
A	*C*	*A*	*B*	0.014	0.159[6]	0.015	0.111[6]	0.005	0.056[6]	0.032	0.182[6]	0.010	0.122[6]
A	*C*	*C*	*A*	0.014	0.025	0.010	—	0.002	—	0.007	—	0.005	0.006
A	*C*	*C*	*B*	0.012	0.016	0.013	0.054[6]	0.004	—	0.010	0.020	0.005	0.025
B	*A*	*A*	*A*	0.078	0.157[6]	0.082	0.059[6]	0.096	0.061[6]	0.099	0.060[6]	0.129	0.083[6]
B	*A*	*A*	*B*	0.066	0.123[6]	0.107	0.206[6]	0.206	0.360[6]	0.144	0.258[6]	0.300	0.234[6]
B	*A*	*C*	*A*	0.066	0.050[6]	0.070	0.154[6]	0.069	0.088[6]	0.031	0.089[6]	0.078	0.100[6]
B	*A*	*C*	*B*	0.055	0.014	0.090	—	0.147	0.047	0.046	—	0.176	0.015
B	*C*	*A*	*A*	0.031	—	0.028	0.011	0.017	—	0.038	—	0.020	0.004
B	*C*	*A*	*B*	0.026	—	0.037	0.007	0.037	—	0.056	0.038	0.033	0.009
B	*C*	*C*	*A*	0.026	0.027	0.024	—	0.012	0.005	0.012	—	0.012	0.008
B	*C*	*C*	*B*	0.022	—	0.031	0.031	0.026	0.050[6]	0.018	—	0.018	0.022
B′	*A*	*A*	*A*	0.090	—	0.060	0.063[6]	0.048	0.015	0.066	0.109[6]	0.068	0.044
B′	*A*	*A*	*B*	0.076	0.063[6]	0.078	0.045	0.103	0.050[6]	0.096	0.075[6]	0.130	0.056[6]
B′	*A*	*C*	*A*	0.076	0.250[6]	0.051	0.122[6]	0.034	0.123[6]	0.021	0.092[6]	0.042	0.148[6]
B′	*A*	*C*	*B*	0.064	0.057[6]	0.065	0.096[6]	0.073	0.090[6]	0.031	0.021	0.077	0.071[6]
B′	*C*	*A*	*A*	0.036	0.016	0.021	—	0.009	—	0.025	—	0.016	0.004
B′	*C*	*A*	*B*	0.030	0.025	0.027	0.015	0.019	0.028	0.037	—	0.020	0.018
B′	*C*	*C*	*A*	0.030	0.018	0.017	—	0.006	—	0.008	—	0.010	0.004
B′	*C*	*C*	*B*	0.026	—	0.022	—	0.013	—	0.012	—	0.012	—

[1]Frequencies were estimated by EH program, not considering allele frequencies lower than 0.05.

[2]Overall = average frequencies across sampled animals.

[3]IF = haplotype frequencies expected under the independence hypothesis.

[4]AF = haplotype frequencies estimated taking association into account.

[5]— indicates haplotype frequencies <0.001.

[6]Indicates AF ≥0.05 in at least one population.

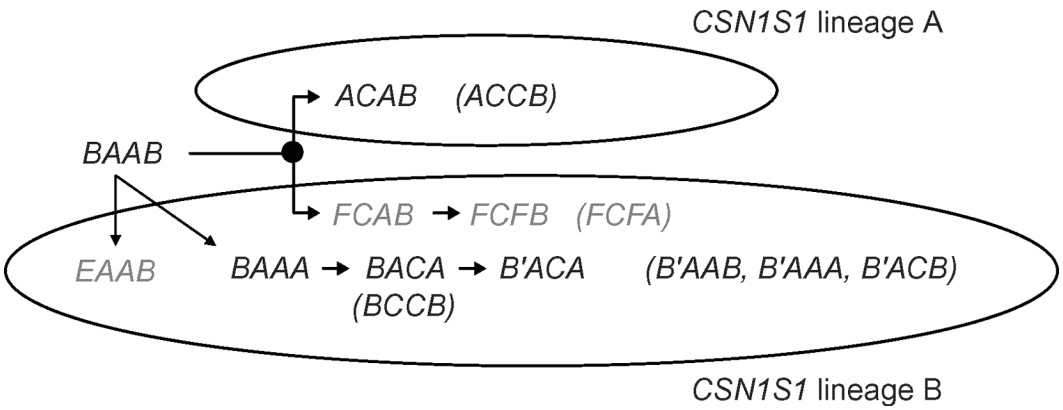

Figure 2. Possible evolution of the casein haplotype. Haplotypes from Caroli et al. (2006) are shown in gray; recombinant haplotypes are given in parentheses. The black dot indicates the probable mutation event leading to the *CSN2*C* allele and to the haplotype *BCAB*.

Linkage disequilibrium occurred in all the populations, with highly significant differences between AF and IF ($P < 0.001$) in Borno (χ^2 value of 80.95, associated with 23 df), Red Sokoto ($\chi^2_{[23]} = 66.71$), WAD Nigeria ($\chi^2_{[23]} = 55.85$), and significant differences ($P < 0.05$) in WAD Cameroon ($\chi^2_{[23]} = 35.27$). Haplotypes *ACAB, BAAB, B'ACA* had much greater AF than IF (Table 2). The opposite was true for haplotypes *BACB, AAAB,* and *AAAA*.

For *CSN2,* the *A* allele was most often associated with *CSN1S1*B* and *CSN1S1*B'* and was almost never linked to *CSN1S1*A* (Table 2). The opposite situation occurred for *CSN2*C,* which was usually associated with *CSN1S1*A,* even if the frequencies of *CSN1S1*B-CSN1S1*B'-CSN2*C* combinations were slightly higher than *CSN1S1*A-CSN2*A*. This finding might indicate that the differentiation from *CSN2*A* to *CSN2*C* occurred before the evolution of *CSN1S1*B* into the numerous mutations affecting the *CSN1S1* locus. This result in West African goats is in agreement with Chessa et al. (2005b) and Caroli et al. (2006). In particular, the *CSN2*A* to *CSN2*C* differentiation might have arisen before the splitting of *CSN1S1* in the 2 lineages (A and B) proposed by Grosclaude et al. (1994). This hypothesis is shown in Figure 2 where a possible evolution is given for the 10 most common haplotypes of the present work, together with other haplotypes considered by Caroli et al. (2006). The haplotype *BCAB,* in which the *CSN2*C* mutation event arose most probably before the *CSN1S1* splitting, is not indicated in Figure 2 because it occurred at a rather low frequency in the 4 populations, whereas the possible recombinant *BCCB* haplotype was more common (Table 2).

The haplotype *FCFA* was common in the northern Italian breeds considered by Caroli et al. (2006) who included it in the proposed phylogeny as a parental haplotype, even if the *CSN3*A* leading mutation was suggested as possible also in *CSN1S1* lineage A, leading to the *ACAA* haplotype. However, the *F-FA* haplotype (the line indicates *CSN2*A* + *CSN2*C*) was found in southern Italian breeds at a rather low frequency (Sacchi et al., 2005), and *F-FB* (named as *F-FD* due to the different *CSN3* nomenclature used) was the most common haplotype, with AF values much higher than IF values. Thus, it can be expected that the high frequency of *FCFA* in Northern Italian populations may be the consequence of a recombinant event in the CN haplotype, which was largely spread in the populations due to either genetic drift or, more intriguingly, to a superior fitness of animals with this haplotype in the specific breeding conditions.

In all cases, it is most probable that the mutation leading to *CSN3*A* occurred neither from *CSN1S1*A* nor *CSN1S1*F,* but from *CSN1S1*B*-carrying haplotypes. The only difference between *CSN3*B* and *CSN3*A* is the AA substitution Ile to Val at position 119 of the mature protein, which is located in CN glycomacropeptide (Yahyaoui et al., 2001; Jann et al., 2004). The antithrombotic and antimicrobical properties of this peptide arising from the κ-CN cleavage by rennet in bovine milk are described (Meisel, 2005; Rhoades et al., 2005). The large diffusion of κ-CN Ile_{119} to Val_{119} substitution in different goat CN haplotypes might be considered in the light of particular biological properties of the CN-glycomacropeptide genetic variants.

CONCLUSIONS

A new allele has been identified and characterized at the *CSN1S1* gene that occurred in all the breeds. No null allele was found in any of the genes analyzed. A high frequency of the *CSN1S1*B, CSN2*A, CSN1S2*A,* and *CSN3*B* alleles, postulated as ancestral in previous

studies, was found in all the populations. The findings suggest a relationship between African goat breeds and the breeds of southern Europe characterized by high frequencies of *CSN1S1* strong alleles, whereas for the other calcium-sensitive CN, clear differences were found in the predominant alleles with respect to European breeds. Interestingly, although *CSN3*B* is usually highly prevalent in *CSN3*A* in European breeds, the *A* and *B* frequencies were more balanced in the African goats.

The CN variability in African goats allowed the inclusion of some haplotypes carrying the *CSN1S1*B* allele and its synonymous variant, *B′*, in the proposal of the CN haplotype phylogenesis. Genetic drift, as well as attractive fitness mechanisms, seems to have strongly influenced the CN haplotype structure in different goat breeds worldwide.

The high frequency of strong alleles in the calcium-sensitive CN as well as the high linkage disequilibrium found among the CN genes in the African breeds analyzed may suggest that specific CN haplotypes have already been selected due to their advantages for nutrition. Haplotypes providing greater protein and CN content would increase the energy content of milk, thus resulting more favorable for growth and survival of young goats and humans consuming the milk.

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