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Goat milk allergenicity as a function of α_{S1}-casein genetic polymorphism

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ABSTRACT

Cow milk allergy is the most frequent allergy in the first years of life. Milk from other mammalian species has been suggested as a possible nutritional alternative to cow milk, but in several cases, the clinical studies showed a high risk of cross-reactivity with cow milk. In the goat species, α_{S1}-casein (α_{S1}-CN), coded by the *CSN1S1* gene, is characterized by extensive qualitative and quantitative polymorphisms. Some alleles are associated with null (i.e., *CSN1S1*0*$_1$) or reduced (i.e., *CSN1S1*F*) expression of the specific protein. The aim of this work was to obtain new information on goat milk and to evaluate its suitability for allergic subjects, depending on the genetic variation at α_{s1}-CN. Individual milk samples from 25 goats with different *CSN1S1* genotypes were analyzed by sodium dodecyl sulfate PAGE and immunoblotting, using monoclonal antibodies specific for bovine α-CN and sera from children allergic to cow milk. A lower reaction was observed to 2 goat milk samples characterized by the *CSN1S1* 0*$_1$*0*$_1$ and *0*$_1$*F* genotypes. Moreover, a fresh food skin prick test, carried out on 6 allergic children, showed the lack of positive reaction to the *0*$_1$*0*$_1$ milk sample and only one weak reactivity to the *0*$_1$*F* sample. The risk of cross-reactivity between cow and goat milk proteins suggests the need for caution before using goat milk for infant formulas. However, we hypothesize that it can be used successfully in the preparation of modified formulas for selected groups of allergic patients. The importance of taking the individual goat CN genetic variation into account in further experimental studies is evident from the results of the present work.

Key words: milk protein, goat, α_{S1}-casein, allergenicity

INTRODUCTION

Cow milk allergy is the most frequent allergy in the first years of life. In the absence of maternal milk, allergic subjects need an alternative protein source, which is usually based on hydrolyzed cow milk proteins (caseins or whey proteins) or soybean-based formula. Milk from various mammalian species (horse, donkey, and goat) has been suggested as a possible alternative to cow milk, but its safety for allergic subjects is still debated.

Even though the results in clinical trials are controversial (Restani, 2004), positive evidence for goat milk tolerance is reported in some papers. For example, a clinical trial performed at Creteil (France) in the 1990s showed that 51 of 55 children with cow milk allergy tolerated goat milk for a feeding period ranging from 8 d to 1 yr (Reinert and Fabre, 1997). In this controversial scenario, differences from the molecular point of view could be responsible for the cross-reactivity or, alternatively, tolerance to goat milk from subjects allergic to cow milk proteins (Restani et al., 2002).

Proteins involved in milk allergies are numerous (Wal, 2002); both CN and whey proteins are involved in sensitization and allergic symptoms. Caseins, and α-CN in particular, are among the most important milk allergens (Docena et al., 1996; Restani et al., 2009). The involvement of each allergenic protein in clinical symptoms could be further influenced by genetic polymorphism, resulting in several variants for each protein (Wal, 2001). These variants are characterized by amino acid exchanges, deletions of peptide fragments, or post-translational modifications such as phosphorylation or glycosylation. All of these modifications may directly influence the protein allergenicity, affecting secondary and tertiary structure of the molecule (Wal, 2001).

Almost 50 genetic variants have been described in bovine milk protein genes (Caroli et al., 2009), and, in the goat, the number of genetic variants is continually rising (Caroli et al., 2007; Marletta et al., 2007). The 6 main goat milk proteins are similar to the correspond-

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ing cow milk proteins in their general classification of α_{S1}-CN, α_{S2}-CN, β-CN, κ-CN, β-LG, and α-LA, but several differences occur between and within species (Caroli et al., 2009). In the goat species, high polymorphism has been found at the 4 CN genes with several alleles associated with null or reduced expression of the specific protein. From a quantitative point of view, the most variable CN gene is *CSN1S1*, coding for α_{S1}-CN. On the basis of α_{S1}-CN content, *CSN1S1* alleles can be classed into 4 groups: strong alleles (*A*, *A'*, B_1, B_2, B_3, B_4, *B'*, *C*, *H*, *L*, and *M*), producing almost 3.5 g/L of α_{S1}-CN each; intermediate alleles (*E* and *I*; 1.1 g/L); weak alleles (*F* and *G*; 0.45 g/L); and null alleles (0_1, 0_2, and *N*), producing no α_{S1}-CN (reviewed in Caroli et al., 2007; Park et al., 2007). In this list, the most recently discovered variant *A'* is included (Küpper et al., 2010). The distribution of these alleles has been investigated in various breeds, showing that the strong alleles have a higher frequency in breeds from the Mediterranean area. This indicates that milk coming from these breeds is more suitable for cheese production, because of the good clotting ability and the better cheese yield associated with the strong alleles. On the contrary, goat milk with low or no α_{S1}-CN has lower curd yield, longer rennet coagulation time, more heat liability, and weaker curd firmness, which also may explain the benefits in digestibility in the human digestive tract (Ambrosoli et al., 1988). The allergenic potency of goat milk with different contents of α_{S1}-CN was investigated in a clinical test carried out on guinea pigs. Goat milk lacking α_{S1}-CN was less allergenic that other goat milk, probably because of a modified β-LG:α_S-CN ratio (Bevilacqua et al., 2001). The occurrence of alleles associated with null or faint content of the different caseins might be exploited for the production of milk with particular nutritional qualities (i.e., hypoallergenic properties for those subjects monosensitized to this protein fraction).

In the search for the molecular aspects involved in the controversial results obtained in clinical studies, the aim of this research was to evaluate if genetic variation at goat α_{S1}-CN could allow the preparation of a hypoallergenic formula suitable for specific subgroups of allergic patients.

MATERIALS AND METHODS

Goat Milk Samples

Twenty-five goats from different breeds (Table 1) were selected in an experimental flock (Borgo Adorno, AL, University of Milan, Italy) on the basis of the *CSN1S1* genotype previously typed according to Caroli et al. (2006). Individual milk samples were collected, frozen at −20°C, and subsequently analyzed by SDS-PAGE (see below for technical details). The milk samples were diluted with distilled water 1:4 (vol/vol); the samples obtained were treated with sample buffer (containing 0.25 m*M* Tris-HCl, pH 6.8; 7.5% glycerol; 2% SDS; and 5% β-mercaptoethanol) at a 1:1 (vol/vol) ratio. Milk total protein content was measured according to Lowry et al. (1951). Relative abundance of α-CN on total protein was calculated on the SDS-PAGE using a gel scanner (Sharp JX-330, Pharmacia Biotech, Cologno Monzese, Milan, Italy) and the Image Master 1D Software. It allows the quantification of proteins by calculating the average density of pixels across the band length and integrating over the bandwidth.

Purified Milk Proteins

Purified cow milk proteins were purchased from Sigma Chemical (St. Louis, MO) at the highest available purification level. All proteins were suspended in sample buffer at a final concentration of 1 mg/mL.

Allergic Subjects

Sera included in this study were from 6 children allergic to cow milk with ages ranging from 0.75 to 9.7 yr; their sensitization patterns at enrollment are reported in Table 2. All 6 children had positive results to the skin prick test (**SPT**) and coated allergen particle test (CAP), and were selected among 150 patients allergic to cow milk on the basis of their strong sensitization to α-CN, which is the reason for the low number of allergic subjects included in the study. The subjects were selected on the basis of their severe clinical reactivity (they showed anaphylaxis) and the specific pattern of sensitization. As shown in Table 2, their reactions were highly positive to caseins in all diagnostic tests, and in particular they were selected only when assigned to classes 5 and 6 (the highest ones) for α-CN in immunoblotting. Informed consent was obtained from the parents of all children participating in this study.

Skin Tests

The fresh food SPT was performed by percutaneous lancing through a drop of goat milk immediately wiped off with absorbent paper using 1-mm tipped lancets (Dome-Hollister-Stier, Slough, UK). A solution of histamine phosphate in 50% of glycosaline (10 mg/mL) and the vehicle were used as positive and negative controls, respectively. Wheal diameters were read through a clear plastic caliper disk scaled in quarter-millimeters under 4 × magnification and recorded as positive when a >3 mm wheal margin (SPT cut-off point) was included within a complete caliper circle,

Table 1. Goat milk samples included in this study with the corresponding breed and genotype at the *CSN1S1* gene, which codes for α_{S1}-CN; total protein content and percentage α-CN of the milk samples are also reported[1]

Sample	Breed	*CSN1S1*	Total protein (g/100 mL)	α-CN (%)
1	**Orobica**	***0$_1$0$_1$***	**2.94**	**21.8**
2	**Frisa**	***0$_1$0$_1$***	**2.98**	**22.2**
3	Frisa	*0$_1$A*	2.61	33.0
4	Frisa	*0$_1$E*	2.09	28.7
5	Saanen	*0$_1$B*	2.37	31.8
6	**Orobica**	***0$_1$F***	**2.39**	**22.5**
7	**Saanen**	***FF***	**3.11**	**22.0**
8	Saanen	*FF*	3.46	23.2
9	Saanen × Camosciata	*AE*	2.97	29.1
10	**Saanen × Camosciata**	***BB***	**3.31**	**31.9**
11	Saanen	*BE*	3.48	26.2
12	Saanen	*EE*	2.73	23.3
13	Saanen × Camosciata	*EF*	2.97	24.4
14	Saanen	*AE*	3.38	28.1
15	**Saanen**	***BE***	**2.91**	**24.8**
16	**Saanen**	***EE***	**3.43**	**26.6**
17	Saanen × Camosciata	*EF*	2.79	24.8
18	Saanen	*AE*	2.88	26.7
19	**Saanen**	***BE***	**2.88**	**23.9**
20	**Saanen**	***EE***	**3.32**	**26.5**
21	Saanen × Camosciata	*AE*	3.28	28.2
22	Saanen	*BE*	3.21	28.9
23	Saanen	*EE*	3.09	25.2
24	Saanen	*EE*	2.80	26.4
25	Saanen	*EE*	3.25	25.9

[1]Samples selected for immunochemical analyses are indicated in bold.

rounding measurements up or down to the nearest 1 mm.

SDS-PAGE

The electrophoretic runs were performed on a gradient polyacrylamide gel with the following characteristics. The gradient running gel contained 12 to 22% of acrylamide; 0.11 to 0.2% of bis-acrylamide; 0.36 *M* Tris-HCl buffer, pH 8.8; 35% of glycerol; 0.1% of SDS; 0.02% of ammonium persulfate; and 0.15% of *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED). The stacking gel contained 3.5% of acrylamide; 0.09% of bis-acrylamide; 0.125 *M* Tris-HCl buffer, pH 6.8; 0.1% of SDS; 0.02% of ammonium persulfate; and 0.15% of TEMED. The running buffer contained 25 m*M* Tris, 0.19 *M* glycine, and 0.1% of SDS (wt/vol), pH 8.8.

After the electrophoretic run (90 V at room temperature, for approximately 6 h), gels were dyed with Coomassie brilliant blue G-250 by the method of Neuhoff et al. (1988). All materials and instruments were purchased from Bio-Rad (Richmond, CA).

Immunoblotting

After SDS-PAGE, proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore,

Table 2. Sensitization patterns to different allergens at enrollment of the 6 subjects allergic to cow milk (milk) included in this study

			SPT[1] (mm)				CAP[2] (kU/L)				IMM[3] (reactivity class)				
Subject	Sex	Age (yr)	α-LA	β-LG	CN	Milk	α-LA	β-LG	CN	Milk	α-LA	β-LG	α-CN	β-CN	Milk
A	Female	1.8	15	11	13	17	22.1	9.39	59.9	70.9	6	3	6	6	6
B	Female	9	6	7	5	5	11.1	6.42	22.1	30.8	3	1	6	6	6
C	Male	4.9	10	4	5	5	6.41	3.08	5.06	7.95	5	3	6	0	6
D	Male	9.7	12.5	0	6	8	ND[4]	ND	ND	ND	0	0	6	2	6
E	Male	2.2	13	7	8.5	7.5	6.22	9.41	11.7	19.4	2	2	5	2	5
F	Female	0.75	14	6	9	7	15.7	1.78	53.8	42.2	0	0	5	0	5

[1]Skin prick test: positive responses have wheal diameter ≥3 mm.

[2]Coated allergen particle test (circulating IgE): positive responses are ≥0.35 kU/L.

[3]Immunoblotting: positive classes are 3 to 6.

[4]Not done due to the lack of serum.

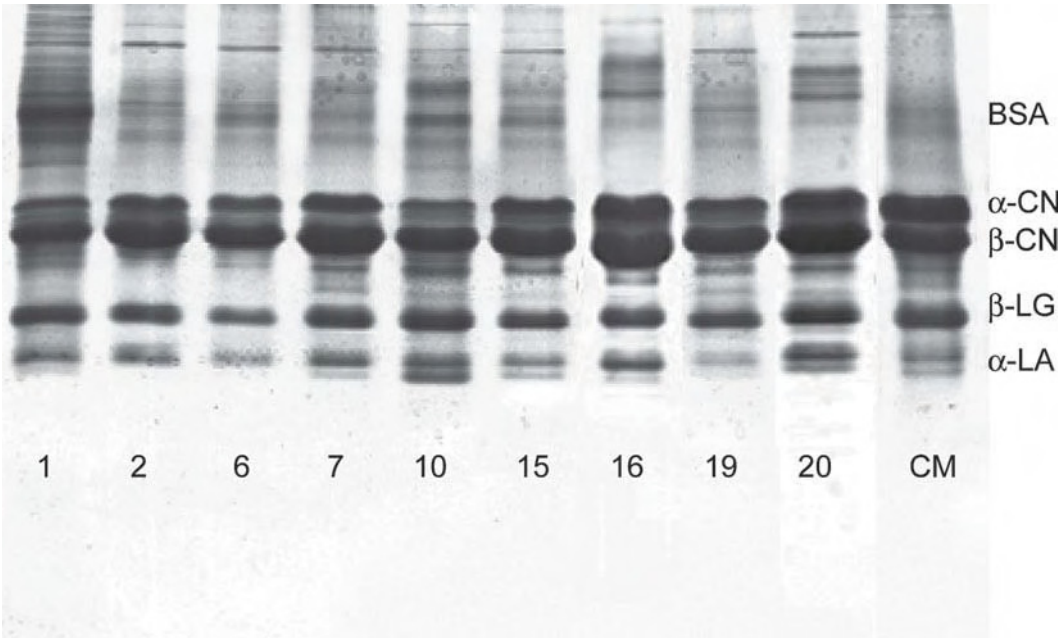

Figure 1. An SDS-PAGE of goat milk samples chosen for immunoblotting. Cow milk (CM) was loaded in parallel. The sample numbers correspond to the following *CSN1S1* genotype: samples 1 and 2 = $0_1 0_1$; 6 = $0_1 F$; 7 = *FF*; 10 = *BB*; 15 and 19 = *BE*; 16 and 20 = *EE*.

Billerica, MA) by Western blotting in a trans-blot electrophoretic transfer cell (Bio-Rad). The membranes were blocked with 1% gelatin and washed 3 times with a 0.25% gelatin solution (150 m*M* NaCl, 5 m*M* Tris, and 0.05% Triton-X) to prevent nonspecific adsorption of the immunological reagents.

The membrane was then immersed in 10 mL of a 0.25% gelatin solution containing 300 μL of serum from subjects allergic to cow milk. Antigen-IgE complexes were detected using 30 μL of goat anti-human IgE polyclonal antibodies labeled with alkaline phosphatase (Sigma-Aldrich, St. Louis, MO).

Finally, after incubation in the bromochloroindolyl phosphate-nitroblue tetrazolium (BCIP/NBT) solution, an intense black-purple precipitate developed at the site of the enzyme binding. The developing solution contained 15% of bromochloroindolyl phosphate and 30% of nitroblue tetrazolium in alkaline phosphatase buffer (100 m*M* Tris, 100 m*M* NaCl, and 5 m*M* MgCl, pH 9.5). Gels and membranes were analyzed using an image scanner and the Image Master 1D Software (Sharp JX-330, Pharmacia Biotech).

RESULTS

On the basis of the 25 goat electrophoretic profiles, we selected 9 samples (1, 2, 6, 7, 10, 15, 16, 19, and 20) in which the α-CN content was noticeably less abundant compared with the abundance of β-CN, to assess their antigenicity with sera from allergic subjects (Figure 1). Figure 2 shows the immunoblottings obtained from incubating the membranes with the sera of 6 subjects allergic to cow milk. The subjects were selected on the basis of their severe clinical reactivity (they showed anaphylaxis) and the highest sensitization patterns for α-CN in immunoblotting (Table 2).

As expected, all subjects reacted strongly to cow α-CN. Considering the goat milk samples, immunoreactivity was found mainly against α-CN (in particular for α_{S2}-CN), but the intensity of positive response was different. The reactivity was lower for samples 2 and 6, which were homozygous and heterozygous for the null *CSN1S1*0*$_1$ allele, respectively. As for goat β-CN, the reactivity was high in subjects A to C, whereas no immunodetection was observed in subject D, as expected on the basis of the subject's sensitization (Table 2). Goat samples 2, 6, and 7 were less antigenic for this subject (Figure 2).

The most interesting goat milk samples (2 and 6) were assayed by fresh food SPT in the same allergic children. Results are illustrated in Table 3. All subjects showed positive results (wheal diameter >3 mm) to cow milk (as expected) and commercial goat milk (except for patient F, wheal diameter = 2 mm). No positive reaction was observed against sample 2 and only one weak reactivity (subject C, wheal diameter = 3 mm) to sample 6, confirming the potential hypoallergenic properties of these goat milk samples.

Table 3. Skin prick test with commercial cow milk, commercial goat milk, and individual goat milk samples 2 and 6 (wheal diameters in mm)[1]

Patient	Cow milk	Commercial goat milk	Goat milk sample 2	Goat milk sample 6
A	10	—	0	0
B	5	3	0	0
C	5	5	2	3
D	7	3	0	0
E	7	3	0	0
F	5	2	0	2

[1]Positive response: wheal diameter ≥3mm; — = not performed.

DISCUSSION

Individual goat milk samples showed SDS-PAGE patterns similar to those of cow milk, except for a lower relative abundance of α-CN. Sera from children highly sensitized to α-CN were used to verify the antigen-antibody complex formation by immunoblotting. Compared with reactions to cow milk, no serum showed a negative reaction pattern, but a lower immunoreaction was found against 2 goat milk samples (samples 2 and 6). This result can be partially explained by the lower amount of α-CN related to the *CSN1S1* genotype of the 2 goats ($0_1 0_1$ and $0_1 F$), also explaining why some allergic children are able to tolerate goat milk, as reported previously (Webber et al., 1989; Ellis et al., 1991). However, the other milk sample homozygous for the null *CSN1S1*$^*0_1$ allele (sample 1) was more immunoreactive than expected on the basis of its *CSN1S1*

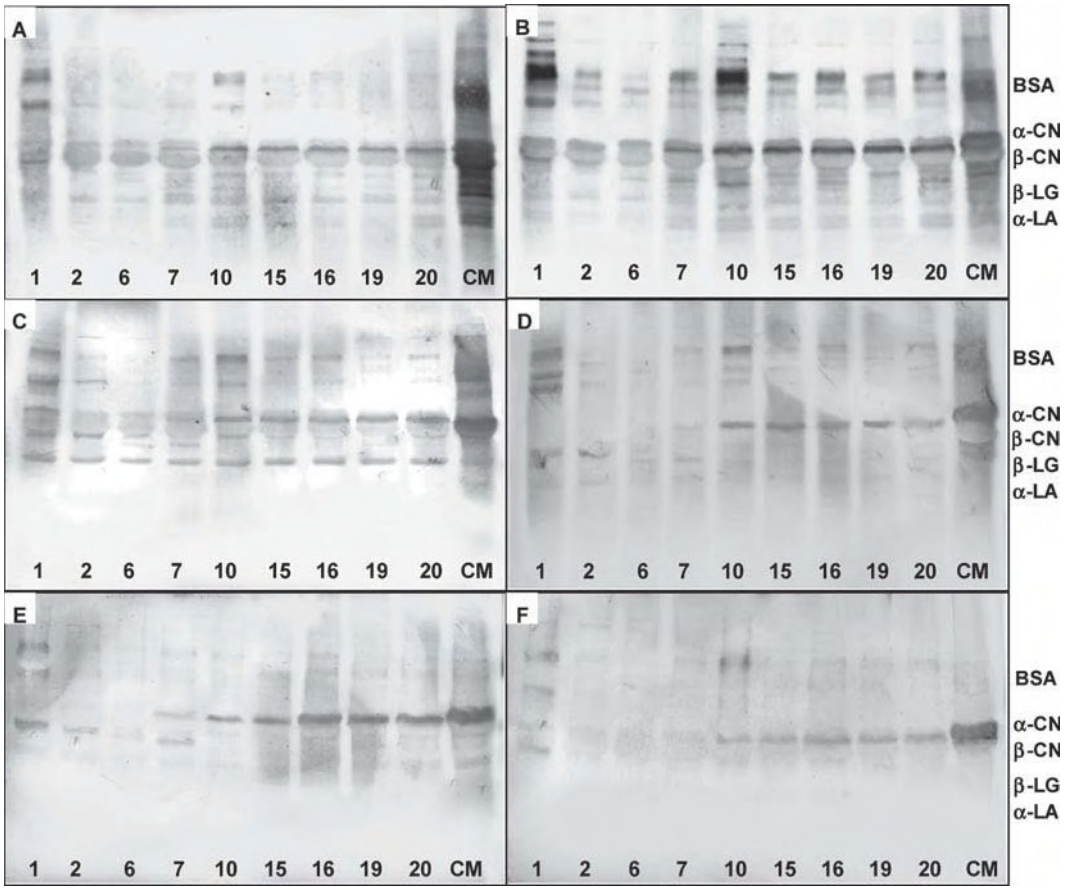

Figure 2. Immunoblotting of selected goat milk samples obtained after incubating the membrane with sera from the allergic subjects A to F (CM = cow milk). The sample numbers correspond to the following *CSN1S1* genotypes: samples 1 and 2 = $0_1 0_1$; 6 = $0_1 F$; 7 = *FF*; 10 = *BB*; 15 and 19 = *BE*; 16 and 20 = *EE*.

genotype, whereas sample 7, homozygous for the "faint" *CSN1S1*F*, also showed a lower reactivity for 1 of the 6 children analyzed by immunoblotting.

The allergenic behavior of the *CSN1S1*E* variant observed in the present trial is noteworthy. An in silico approach was used by Chessa et al. (2008) to find IgE-binding epitopes already identified in the bovine species within the goat CN sequences, with particular attention to the occurrence of genetic polymorphism at *CSN1S1*. Differences in the AA sequence were found in 7 genetic variants. Apart from the fifth (YPSGAWYYVPLGTQY) and the first minor (ELSKDIGSES) epitopes, for which no variation occurred between bovine and goat *CSN1S1*, several differences were found both between the 2 species and among goat *CSN1S1* alleles with a total of 17 exchanged AA and 22 deleted AA, 21 of which involved *CSN1S1*E*. In the present study, no samples carrying this allele, even if in the homozygous state or heterozygous with a null or faint allele, showed a lower allergenic reactivity expected on the basis of the intermediate α_{s1}-CN content associated with *CSN1S1*E*. This intriguing aspect might be partially explained by 2 AA substitutions occurring in this variant at the level of the second and sixth major epitopes, possibly increasing its allergenicity. This hypothesis needs to be confirmed.

The identification of a more suitable protein source for cow milk-allergic children represents an important goal for pediatricians and nutritionists. However, the best alternative diet for children with an allergy to cow milk proteins remains to be defined, at least for subjects with a specific pattern of sensitization (Docena et al., 1996; Restani et al., 2009). Data from the present study, including immunoblotting and SPT results, suggest that goat milk from a particular *CSN1S1* genotype could be used as an alternative protein source for the production of hypoallergenic formulas in the case of specific α-CN sensitization. However, the differences found in the allergic reactions to goat milk with the same null *CSN1S1* genotypes, as well as the similar lower reactivity exerted by the full fat milk sample, indicate that milk allergenicity could be affected by other factors that still need to be investigated and understood. In the case of goats with reduced amount of α_{S2}-CN, the small decrease in the allergenic potency of the CN fraction suggested the crucial role of whey in the allergic reaction (Marletta et al., 2004). Similarly, interactions with whey protein components or other CN fractions might be the cause of the individual differences found among the goat milk samples analyzed. Even if it was not possible to split α-CN into the 2 fractions α_{S1}-CN and α_{S2}-CN due to the very close migration on SDS-PAGE, we believe that the immunoblotting results, supported by STP data, are suggestive proof that particular *CSN1S1* genotypes can reduce the intolerance of allergic subjects in specific cases. Because the potential hypoallergenic properties were found for only 2 of the 10 milk goat samples carrying null or weak *CSN1S1* genotypes, further studies are needed to explain the particular behavior of these 2 samples, to clearly assess the relations between goat CN genotypes and milk protein tolerability. Nevertheless, these preliminary results are of interest, because they could explain the controversial literature on goat milk tolerability.

In all cases, the lower cross-reactivity of some children to a particular goat's milk does not guarantee that this milk can be considered as a safe protein source for allergic subjects. The severity of goat milk allergic reactions observed in some children indicates that great caution is needed before considering goat milk as a suitable and safe substitute for feeding children at atopy risk. This cautious approach does not rule out further investigations about goat milk tolerance in subjects allergic to cow milk. The reasons for the lack of clinical cross-reactivity of these children should be established and new dietary applications should be found for goat milk (Restani, 2004).

In conclusion, the use of goat milk for infant formulas might be suggested only in well-selected cases and after appropriate nutritional modifications. In fact, although the use of goat milk in hypoallergenic formulas is not generally recommended, positive results could be reached in the preparation of modified formulas (as such or hydrolyzed) suitable for defined groups of allergic patients (Restani et al., 1999).

The importance of taking individual goat CN genetic variation into account in further studies is evident from the results of the present work. Selection and breeding of particular goat genetic lines could be performed for the production of milk devoted to the preparation of infant formulas. However, the genetic information on goat *CSN1S1* is not sufficient to select the animals suitable for the creation of these hypoallergenic goat milk lines. The allergenicity of milk should be assessed by appropriate clinical studies before selecting the animals within a certain group of *CSN1S1* genotypes (i.e., $0_1 0_1$ and $0_1 F$). Moreover, all CN genes should be considered to fix the most hypoallergenic CN combinations in these hypoallergenic lines. This scientific approach is complicated by the high variation occurring at all CN genes, which requires many genetic and clinical tests before selecting and breeding the most suitable goats for safe specific infant formulas. Nevertheless, significant advantages could derive from such studies from the point of view of human nutrition and for the defense and valorization of goat genetic resources.

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REFERENCES

Ambrosoli, R., L. Di Stasio, and P. Mazzocco. 1988. Content of α_{S1}-casein and coagulation properties in goat milk. J. Dairy Sci. 71:24–28.

Bevilacqua, C., P. Martin, C. Candalh, J. Fauquant, M. Piot, A. M. Roucayrol, F. Pilla, and M. Heyman. 2001. Goats' milk of defective α_{S1}-casein genotype decreases intestinal and systemic sensitization to β-lactoglobulin in guinea pigs. J. Dairy Res. 68:217–227.

Caroli, A., F. Chiatti, S. Chessa, D. Rignanese, P. Bolla, and G. Pagnacco. 2006. Focusing on the goat casein gene complex. J. Dairy Sci. 89:3178–3187.

Caroli, A., F. Chiatti, S. Chessa, D. Rignanese, E. Ibeagha-Awemu, and G. Erhardt. 2007. Characterization of the casein gene complex in West African goats and description of a new α_{S1}-casein polymorphism. J. Dairy Sci. 90:2989–2996.

Caroli, A. M., S. Chessa, and G. J. Erhardt. 2009. Invited review: Milk protein genetic variation in cattle: Impact on animal breeding and human nutrition. J. Dairy Sci. 92:5335–5352.

Chessa, S., F. Chiatti, D. Rignanese, G. Ceriotti, A. M. Caroli, and G. Pagnacco. 2008. Analisi in silico delle sequenze caseiniche caprine. Sci. Tecn. Latt. Cas. 59:71–79.

Docena, G. H., R. Fernandez, F. G. Chirdo, and C. A. Fossati. 1996. Identification of casein as the major allergenic and antigenic protein of cow's milk. Allergy 51:412–416.

Ellis, M. H., J. Short, and D. C. Heiner. 1991. Anaphylaxis after ingestion of a recently introduced hydrolyzed whey protein formula. J. Pediatr. 118:74–77.

EFSA Journal. 2004.Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of goats' milk protein as a protein source for infant formulae and follow-on formulae. 30:1–15.

Küpper, J., S. Chessa, D. Rignanese, A. Caroli, and G. Erhardt. 2010. Divergence at the casein haplotypes in dairy and meat goat breeds. J. Dairy Res. 77:56–62.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin Phenol reagent. J. Biol. Chem. 193:265–275.

Marletta, D., S. Bordonaro, A. M. Guastella, P. Falagiani, N. Crimi, and G. D'Urso. 2004. Goat milk with different αs_2-casein content: Analysis of allergenic potency by REAST-inhibition assay. Small Rumin. Res. 52:19–24.

Marletta, D., A. Criscione, S. Bordonaro, A. M. Guastella, and G. D'Urso. 2007. Casein polymorphism in goat's milk. Lait 87:491–504.

Neuhoff, V., N. Arold, D. Taube, and W. Ehrhardt. 1988. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie brilliant blue G-250 and R-250. Electrophoresis 9:255–262.

Park, Y. W., M. Juárez, M. Ramos, and G. F. W. Haenlein. 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68:88–113.

Reinert, P., and A. Fabre. A. 1997. Utilisation du lait de chèvre chez l'enfant. Expérience de Creteil. Pages 119–121 in Proc. Colloque Intérets Nutritionnel et Diététique du Lait de Chèvre, 7th November 1996, Niort, France. INRA, Paris, France.

Restani, P. 2004. Goat milk allergenicity. J. Pediatr. Gastroenterol. Nutr. 39:323–324.

Restani, P., C. Ballabio, C. Di Lorenzo, S. Tripodi, and A. Fiocchi. 2009. Molecular aspects of milk allergens and their role in clinical events. Anal. Bioanal. Chem. 395:47–56.

Restani, P., B. Beretta, A. Fiocchi, C. Ballabio, and C. L. Galli. 2002. Cross-reactivity between mammalian proteins. Ann. Allergy Asthma Immunol. 89:11–15.

Restani, P., A. Gaiaschi, A. Plebani, B. Beretta, G. Cavagni, A. Fiocchi, C. Poiesi, T. Velonà, A. G. Ugazio, and C. L. Galli. 1999. Cross-reactivity between milk proteins from different animal species. Clin. Exp. Allergy 29:997–1004.

Wal, J. M. 2001. Structure and function of milk allergens. Allergy 56(Suppl. 67):35–38.

Wal, J. M. 2002. Cow's milk proteins allergens . Ann. Allergy Asthma Immunol. 89:3–10.

Webber, S. A., R. A. C. Graham-Brown, P. E. Hutchinson, and D. A. Burns. 1989. Dietary manipulation in childhood atopic dermatitis. Br. J. Dermatol. 121:91–98.