Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Animal Breeding and Genetics

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Table of contents

Abstract

The total number of goats (*Capra hircus*) in Sweden is small compared to the Swedish population of cattle and the Norwegian population of goats. Cheese making from goat milk is one of the productions done in Sweden, even though Swedish goats have a low percentage of caseins in the milk (2.1 %) compared to Nubian goats. Caseins are one factor that affect the produced cheese yield from milk. There are four different caseins; α_{s1}-casein (α_{s1}-CN), α_{S2}-casein (α_{s2}-CN), β-casein and κ-casein. Their respective coding genes are CSN1S1, CSN1S2, CSN2 and CSN3. This study focused on α_{s1}-CN and CSN1S1 with the exon 12 due to a deletion in this exon that has been reported at a high frequency (67.6 %) in Norwegian goats. Forty-eight individual goats of the breeds Swedish Landrace goat, Jämt goat, Lapp goat and Göinge goat from all around Sweden were sequenced for exon 12 to be analysed if they carry the Norwegian deletion. In addition, milk samples from some of these goats and bulk milk have been analysed for the proportions of proteins and caseins. The results showed that the deletion did exist in all the Swedish breeds mentioned above and the allele frequency for the deletion was 94.1 %. The alleles G' and A' were also detected in the target region and the analysed milk samples showed that 53 % had low expression. Overall, this study gives insights about the deletion occurred at exon 12 of the CSN1S1, which affects the α_{s1}-CN protein.

Sammanfattning

Det totala antalet getter (*Capra hircus)* i Sverige är lågt jämfört med den svenska populationen av nötkreatur och den norska populationen av getter. Ostproduktion av getmjölk är en av de produktioner man använder getter till i Sverige, även om svenska getter har en låg procentandel av kasein i mjölken (2,1%) jämfört med nubiska getter. Kaseiner är en faktor som påverkar ostutbytet från mjölk. Det finns fyra olika kaseiner; α_{s1}-kasein (α_{s1}-CN), α_{s2}-kasein (α_{s2}-CN), β-kasein och κ-kasein. Deras respektive kodande gener är CSN1S1, CSN1S2, CSN2 och CSN3. Denna studie fokuserade på α_{s1}-CN och CSN1S1 med exon 12 på grund av en deletion i detta exon som har rapporterats vid en hög frekvens (67,6%) i norska getter. Ett antal av 48 enskilda getter av rasen svensk lantrasget, Jämtget, Lappget och Göingeget från hela Sverige har sekvenserats för exon 12 och analyseras för om de har den norska deletionen. Dessutom har mjölkprov från några av dessa getter och tankmjölk analyserats för proportionerna av proteiner och kaseiner. Resultaten visade att deletionen fanns i alla ovan nämnda svenska raser och allelfrekvensen för deletionen var 94,1%. Allelerna G' och A' upptäcktes även i målområdet och de analyserade mjölkproverna visade att 53% hade lågt uttryck. Sammantaget ger denna studie insikter om deletionen i exon 12 av CSN1S1 som påverkar as1-CN-proteinet.

Introduction

In Sweden, the total number of goats (*Capra hircus*) is small compared to the number of cattle and the Norwegian goat population. The total number of goats in Sweden year 2016 was 15,202 (Hemlin, 2018) and that can be both production animals and animals in zoos. In comparison, there were 68,561 goats in total in Norway the same year (FAOSTAT, 2017a) but only 34,862 dairy goats (Statista, 2018) and the total number of cattle in Sweden was 1,501,345 in the year 2017 (Grönvall, 2017).

The production of goat milk yield has increased since the 1970th to 2016 in the European Union (FAOSTAT, 2017b) but goat milk is still regarded as a niche product and are not common in the mass- market (Solaiman, 2010). Goat milk products such as cheese, have become a great interest in several developed countries (Haenlein, 2004). The production and consumption of goat cheeses in Sweden is increasing in interest due to an increasing trend in locally produced food (Johansson *et al*., 2015) and there were 4294 farmers that had reported they had some sort of goat production in the year of 2016 (Hemlin, 2018). For the breed Swedish Landrace goat, it was 45 farmers with 2,600 goats that had applied for environmental allowance of conservation of threatened livestock breeds in the year of 2013 (Smaka Sverige, 2015). Other Swedish breeds are Jämt goat, Lapp goat and Göinge goat and together they are called Allmoge goats. According to Allmogegeten (2017), the population was 487 animals in 79 herds for Jämt goats, 259 animals in 56 herds for Lapp goats and 424 animals in 88 herds for Göinge goats in the end of the year 2015, in Sweden. All these four breeds are included in this study.

Casein as an important factor in cheese production

The amount of total solids such as fat, protein and casein in the milk decides the amount of resulting cheese (Soryal *et al*., 2005). Especially the casein content of the milk is important since it influences the rheological properties of the rennet gel. Its setting speed and maximum firmness is also influenced of the casein content (Park *et al*., 2007). Casein is the largest part of the protein in the milk and the casein content is about 80% of all the proteins in goat milk in general (Dagnachew & Ådnøy, 2014) but only 72% in Swedish Landrace goat (Högberg, 2011). The other proteins are α- lactalbumin and β- lactoglobulin (Martin *et al*., 2002). In goat milk in general, the protein content is 4.6% (Park *et al*., 2007). The casein level depends on for example fluctuation during the lactation period and also varying in different breeds. During lactation, the level both decreases and increases (Soryal *et al*., 2005). In a study of Soryal *et al*. (2005) the casein level started high in the beginning, in May, and then decreased towards the middle of the lactation. The level then raised and later decreased in the middle. It reached its highest peak in October, right before the dry period (Soryal *et al*., 2005).

Soryal *et al*. (2005) did also compare different breeds in casein level of the milk. The breeds were the Nubian goat and the Alpine goat. The comparison resulted in that the Nubian goat had 3.47% casein in the milk while the Alpine goat had 2.20%. Higher levels of caseins get higher cheese yield. From 10 kg milk from the Nubian goat resulted in 2.71 kg cheese and from the Alpine goat it resulted in 1.69 kg cheese (Soryal *et al*., 2005). The Swedish Landrace goat had 2.10% casein in the milk (Högberg, 2011). The amount of cheese from 10 kg milk from the Swedish Landrace goat is still not scientifically tested but Oviken ost says that it produces 0.8-1 kg cheese from 10 liters of milk (Oviken Ost, place in SWEDEN). That could

mean that the milk from the Nubian goat results in higher cheese yield than from the Swedish Landrace goat.

Aim of this study

Given that the main goal in goat production in Sweden today is cheese production and that the casein content in the Swedish Landrace goat is low and gives a low cheese yield compared to the Nubian breed. This study aims to investigate the exon 12 in the casein gene CSN1S1 with focus on a specific locus in the Swedish Landrace goat, Jämt goat, Lapp goat and Göinge goat. Johansson *et al*. 2014 reported that there is a need for genotyping Scandinavian goats and start to breed for the genotype, which is one factor to give a higher cheese yield because of the negative economic consequence of the high frequencies of low yielding genotypes. Genetic variation in the exon 12 in CSN1S1, with focus on the specific locus, between the four different breeds will also be observed in the current study. Genetic differences outside the exon is not analysed in this study.

Literature study

Four different casein proteins

There are four types of casein proteins in goat milk; α_{s1}-casein (α_{s1}-CN), α_{s2}-casein (α_{s2}-CN), β-casein and κ-casein (Martin *et al*., 2002). They have different concentration in the milk of all proteins and caseins but differences occur between breeds and different studies as can be seen in table 1 (Damián *et al*., 2008; Selvaggi *et al*., 2014). Each type has their own coding gene; CSN1S1 (α_{s1}-CN), CSN1S2 (α_{s2}-CN), CSN2 (β-casein) and CSN3 (κ-casein) (Vacca *et al*., 2014). These genes are placed in a cluster within a length of 250 kilo base pairs (kb) on the chromosome six in goats but in another order than mentioned above: CSN1S1, CSN2, CSN1S2 and CSN3 (Martin *et al*., 2002; Rijnkels, 2002). The 250 kb part of the genome shows a below-average level of repeats (Rijnkels, 2002). The genes have different sizes were CSN1S1 in goats is 17.5 kb, CSN2 is 8.5 kb, CSN1S2 is 18.5 kb and CSN3 is 13 kb long (Martin *et al*., 2002).

Table 1. The proportions in % for the caseins of all caseins for the two breeds Saanen and Anglo-Nubian in Uruguay (Damián *et al*., 2008) and from Selvaggi *et al*. (2014)

Casein	**Saanen**	**Anglo-Nubian**	**Selvaggi *et al*.**
α_{s1}-CN	10.7±1.48	25.0±1.52	5.60
α_{s2}-CN	16.1±1.05	10.6±0.95	19.20
β-casein	56.9±0.97	49.3±1.2	54.80
κ-casein	16.3±0.57	15.0±0.76	20.40

The role of the caseins

Casein works as a transporter of protein, calcium and phosphate from the mammary gland to the newborn kid. In the milk, the caseins are found in the form of micelles with diameters between 150 – 300 nm. They are in different sizes and forms and roughly spherical. The formation of the micelle is depending on calcium (Müller-Buschbaum *et al*., 2007) and its properties are different in goats and cows (Park *et al*., 2007). The size of the micellar is assumed to be negatively correlated to all milk components (Mestawet *et al*., 2013) but not significantly correlated with only casein amount, which is 0.23, in Swedish cows (Glantz *et al*., 2010). Glantz *et al*. (2010) notes that with smaller micellar sizes comes firmer and more compact gel network of the coagulated milk and gives the gel a better quality. The goat casein micelles are less heat stable, loses the β-casein more easily and contains more inorganic phosphate and calcium than cow casein micelles (Park *et al*., 2007). The caseins are divided into calcium-sensitive and non-calcium-sensitive, calcium inhibits or prevent the precipitation of the calcium-sensitive caseins in mixtures (Müller-Buschbaum *et al*., 2007).

While CSN1S1, CSN2 and CSN1S2 are evolutionary related and coding for calcium-sensitive caseins, CSN3 is included in the genetic cluster and physically close to the other caseins. The function of CSN3 is to stabilize the micelles of the calcium-sensitive caseins (Rijnkels, 2002). For the different casein genes, there are several polymorphisms that code for different amounts of casein in the milk (Park *et al*., 2007). The function of the casein genes seems to have a small structure requirement and that could be the reason to that casein genes are rapidly

evolving (Prinzenberg *et al*., 2005). CSN1S1 is the most studied casein gene and are interesting in both goats and cows (Park *et al*., 2007).

CSN1S1

α_{s1}-CN has a key role in the cheese curd formation due to its special micelle structure. Because, compared to the other calcium- sensitive caseins, α_{s1}-CN is more phosphorylated and in the presence of calcium it gets greater solubility (Selvaggi *et al*., 2014).

SNPs

Hayes *et al*. (2006) found 39 single nucleotide polymorphisms (SNPs) with different alleles within all the casein genes in Norwegian goats. These were in exons, introns and promoter regions and most of the SNPs were within CSN1S1 and CSN3 (Hayes *et al*., 2006).

The gene CSN1S1 has 19 exons for goats (NCBI, 2017) and on six of them there have been SNP markers described (Hayes *et al*., 2006). Alleles found on this gene have been increasing through years (Grosclaude *et al*., 1987; Dagnachew *et al*., 2011; Vacca *et al*., 2014) but in the year of 2014 the number were 18 alleles found on the gene in goats; A, B1, B2, B3, B4, B', C, D, E, F, G, H, I, L, M, N, 01 and 02. The gene expressions of these are null (N, 01 and 02), weak (D, F and G), intermediate (E and I) or strong (A, B1, B2, B3, B4, B', C, H, L and M) (Vacca *et al*., 2014). The null alleles give no production of α_{s1}-CN in goat milk but the weak gives 0.45 g/l milk, intermediate 1.1 – 1.7 g/l and strong 3.5 g/l (Park *et al*., 2007).

The null and weak alleles are at low frequencies in most European breeds (Devold *et al*., 2011). In France, selection on genetics in milk protein composition has occurred and been developed during the last 30 years with focus on α_{s1}-CN and to increase the frequencies of alleles that gives most casein production. The breeds Alpine and Saanen are two of these breeds where this selection has occurred and these breeds showed in both the years of 2004 and 2012 in Italy low frequencies of null alleles of α_{s1}-CN (Frattini *et al*., 2014). Also the breed Sarda in Italy had low frequency of null alleles, in the year of 2014 (Vacca *et al*., 2014).

A deletion in the Norwegian goats

A mutation in exon 12 on this gene has been found in Norwegian goats. This mutation is a deletion and gives very low gene expression, which leads to a decrease in dry matter in the milk (Hayes *et al*., 2006). The reason to the low gene expression is that the protein gets truncated with the deletion (Berget *et al*., 2010) but there is no information about changes in the amino acid chain or protein folding with this deletion. Hayes *et al*. (2006) was first to report about this deletion after Ådnøy *et al*. (2003) presented the high frequency of 0- alleles in the CSN1S1 gene in Norwegian dairy goats in the northern Norway. The deletion, also classified as a SNP marker (Hayes *et al*., 2006), had the high frequency of 0.86 in the dairy goats in farms in northern Norway in the year of 2003 (Ådnøy *et al*., 2003). This frequency is unexpected because the breeding goal in Norway has for a long time focused on higher dry matter content in the milk (Hayes *et al*., 2006). In this SNP on exon 12, there can be three different alleles; the new allele that is the deletion (D'), G' or A' with the frequencies 0.676, 0.129 and 0.195 respectively (Dagnachew & Ådnøy, 2014). The sequences for the alleles and the deletion are presented in table 2. The frequency for D' has changed from 0.860 in the year 2003, 0.745 in the year 2006 (Hayes *et al*., 2006), 0.737 (Dagnachew *et al*., 2011) to the number Dagnachew & Ådnøy (2014) reported (0.676) so it has been a decrease of the mutation in the Norwegian goat population.

Table 2. The DNA- sequences for the three polymorphisms were the deletion on exon 12 is located in Norwegian goats (Dagnachew & Ådnøy, 2014)

Allele	Sequence
D'	CTGAAAAATAC
G'	CTGAAGAAATAC
A'	CTGAAAAAATAC

The homozygosity of the allele D' gave high milk yield in kg and free fatty acids (FFA) but low protein, fat and lactose percentage. Homozygosity of the other alleles gave the opposite effect except for protein percentage for G'/G' that had low effect. Heterozygosity with the allele D' gave similar effect as the homozygosity of D' and heterozygosity with G'/D' gave low milk yield and FFA but high percentage of protein, fat and lactose. The deletion on exon 12 also gave a strong rancid taste on the milk (Dagnachew *et al*., 2011). Skeie *et al*. (2014) reported that cheese made of milk from goats that were homozygous for the deletion had more rancid flavour than from goats that were heterozygous for the deletion. More about the flavour in the milk and cheese further down.

This deletion could be present in Swedish goats as well due to the short distance between Norway and Sweden and that the goats could have common ancestors. In a study of Johansson *et al*. (2014) there were 65 % of Swedish goats that had a low expression of α_{s1}-CN (0 - 6.9 % of all caseins) and this result is similar to the result in Norwegian goats. The allele D' at exon 12 on CSN1S1 in Norwegian goats was 67.6% in the study of Dagnachew & Ådnøy (2014). Together with another deletion located at exon 9, D' cause a high frequency of null allele at the CSN1S1-gene (Dagnachew & Ådnøy, 2014).

Haplotypes

When it comes to haplotypes, both CSN1S1 and CSN3 had significant effects on milk production traits and overall both genes had significant haplotype effects on protein percentage (Hayes *et al*., 2006; Dagnachew & Ådnøy, 2014). Ten haplotypes have been found associated with the CSN1S1 gene and were significant in the effect of protein percentage, fat percentage and fat kg (Hayes *et al*., 2006). In the study of Hayes *et al*. (2006), the haplotype with the highest frequency (0.72) in CSN1S1 had negative effect on those factors while another haplotype with the frequency of 0.06 hade the largest positive effects on both protein and fat percentage.

Dagnachew & Ådnøy (2014) on the other hand found six haplotypes on CSN1S1 where the most frequent (0.667) and two others did affect protein percentage significantly negative. There were also two haplotypes on the same gene that had significant positive effect on protein percentage. In general, the haplotypes on CSN1S1 had significant effects on FFA, fat percentage, protein percentage and milk yield in kg (Dagnachew & Ådnøy, 2014).

Why Hayes *et al*. (2006) and Dagnachew & Ådnøy (2014) analysed and reported different numbers of haplotypes can be because they used different methods for frequencies. They had frequencies >4 and >0.01 respectively. The frequency >4 can mean that Hayes *et al*. (2006) only reported haplotypes that were found in more than four individuals but Dagnachew & Ådnøy (2014) mean that the frequency of haplotypes is in each locus.

Flavour in milk and cheese

An important factor for the consumer of the goat cheese is flavour and that is mostly influenced by the amount of FFA in the milk (Soryal *et al*., 2005). CSN1S1 does not only

code for casein but also FFA in the milk. The FFA are released from fat molecules and gives an unpleasant flavour to the milk. The different alleles of CSN1S1 can have an important role in the FFA concentration in the milk. This due to that it has been found that high levels of FFA in the milk are related to the "weak" alleles of the gene. How strong the milk flavour is, in this case rancid, followed the same curve as the FFA content in the milk through different SNP markers on all the casein genes (Dagnachew *et al*., 2011).

CSN1S2

α_{s2}-CN is not investigated that much compared to the other caseins which could be because it is difficult to isolate and for doing purification. The peptides of α_{s2}-CN are very phosphorylated and the casein can in few cases reach up to the content of 29% of all caseins in the milk. The micelles of the casein in those cases were bigger than normal (Selvaggi *et al*., 2014).

The gene CSN1S2 has 18 exons and their length varies between 21 – 266 base pairs (bp). There are eight alleles for CSN1S2; A, B, C, E, F and G that relates to normal amount of α_{s2}-CN, D that codes for low amount and O that codes for null. D is a rare and defective variant of α_{s2}-CN and is a deletion on exon 11 and the intron after. A normal amount of α_{s2}-CN is about 2.5 g/l milk (Marletta *et al*., 2007).

Hayes *et al*. (2006) reported that there were five SNPs in the exon and intron regions, one in exon three, one in exon 15, two in intron 15 and one in intron 16 in CSN1S2 in Norwegian goats. Dagnachew *et al*. (2011) and Dagnachew & Ådnøy (2014) also reported that there were five SNPs in CSN1S2 but only used four of them; one in exon three and three in exon 16. The SNP in exon 15 were not used in their studies and the SNPs in the intron 15 became the exon 16 instead. The most frequent allele (95%) on the first SNP on exon 16 accounted for a significantly low milk yield. It was the opposite for the last two SNPs located on exon 16. They had a significant effect for high milk production in kg but protein percentage was significant negatively affected from the same SNPs (Dagnachew *et al*., 2011). Four unique haplotypes for CSN1S2 have been found (Hayes *et al*., 2006) but there are no significant effects of the haplotypes in Norwegian goats for either protein percentage, fat percentage or FFA concentrations in the milk (Dagnachew & Ådnøy, 2014).

CSN2

β-casein is the most lavish protein in goat milk and is important in the curd formation of the milk in the presence of chymosin because of that β-casein is a part of determining the properties of the surface of the micelles. It has been shown that without β-casein in the milk, the coagulation time gets longer and the curd firmness decreases. The cheese yield was 80% of normal milk when β-casein was absent (Selvaggi *et al*., 2014).

CSN2 has nine exons that has a length of 24 – 492 bp (Dagnachew *et al*., 2011) and there have been found six SNPs on the gene mostly in promoter region but one in exon seven (Dagnachew et al., 2011; Dagnachew & Ådnøy, 2014). The gene has eight alleles: A, A1, B, C, D, and E that are associated to normal amount of β-casein and O and O' that are associated to null amount of β-casein. These two null alleles come from two different mutations that gives premature stop codons in the exon seven of CSN2. The curd firmness gets weaker and longer rennet coagulation time occur when a null allele is present in β-casein than in normal milk. The allele C is the most frequent in Italian breed (Selvaggi *et al*., 2014).

Hayes *et al*. (2006) found six unique haplotypes for CSN2 but both Hayes *et al*. (2006) and Dagnachew & Ådnøy (2014) analysed four haplotypes in Norwegian goats. They were however not significant for effects on protein or fat percentage and not either on milk production in kg and FFA (Hayes *et al*., 2006; Dagnachew & Ådnøy, 2014). The number of haplotypes can be different because the both authors used two different systems in frequencies mentioned earlier.

CSN3

The role of κ-casein

κ-casein is the only one among the caseins that is soluble in the presence of calcium ions. The phosphate component of the κ-casein is also much smaller compared to other caseins and there has been an increasing interest of κ-casein due to the role it has on technological properties on the milk (Selvaggi *et al*., 2014). The gene expression of CSN3 stabilize and regulate the micelle formation (Caravaca *et al*., 2009) and κ-casein is, unlike the other caseins, hairs on the micelles of the calcium- sensitive casein and is protecting them. This is only occurring in the right pH. When the pH is disturbed, the micelles of calcium-sensitive caseins is unprotected (Vasbinder *et al*., 2003). The right pH in this case is 6.5. At this pH, the chymosin can cleave κ-casein and produce para-κ-casein that is insoluble. The para-κ-casein remains on the surface of the micelles and make them to clot but only if the κ-casein can bind to the calcium-caseins in the core of the micelles (Creamer *et al*., 1998).

Significant effects on κ-casein variants

CSN3 is almost as polymorphic as CSN1S1 and has 16 alleles; A, B, B', B'', C, C', D, E, F, G, H, I, J, K, L and M. A and B are the most frequent alleles in the breeds in Spain, France, Italy and Egypt, the F allele is found only in wild breeds, G only in Italian breeds in high frequencies and the alleles C, D, H, I, K, L and M in intermediate or low frequencies in few breeds. The alleles A and B effect the casein and protein levels in milk differently (Selvaggi *et al*., 2014). Heterozygotes with these alleles and homozygotes for B associates with higher casein and protein level than the homozygote for A in a Spanish breed and the genotypes of BB and AB had the highest frequencies in CSN3 in this breed (Caravaca *et al*., 2009). The rennet coagulation time (in cheesemaking) was significantly affected by CSN3 and the homozygote for B was better than the heterozygote with A and B in the same Spanish breed (Caravaca *et al*., 2011). Chiatti et al. (2007) also reported that the homozygote of B and heterozygote of A and B significantly get higher casein percentage, but in an Italian breed.

SNPs

12 SNPs were found in CSN3 in Norwegian goats and had most SNPs with biggest frequencies of the rare alleles. All SNPs were in a cluster in the promoter region of the gene (Hayes *et al*., 2006). In a later study, 14 SNPs were found were one of them were in exon four (Dagnachew & Ådnøy, 2014). Six SNPs were found for having significant effects on protein percentage in the milk and they were called 27, 31, 33, 34, 36 and 37. The four first SNPs were significant for high percentage and the two last for low percentage. For fat percentage there were only one significant SNP, number 34 and it was for high percentage. The trait milk production in kg were significantly affected of four SNP: 28, 34, 36 and 37. The first two for low kg and the other two for high kg. All these SNPs with significantly effects on protein and fat percentage and milk production in kg had the higher frequencies for the rare alleles in comparison with the other SNPs on CSN3. The frequencies were: 27- 0.421, 28- 0.493, 31- 0.466, 33- 0.465, 34- 0.480, 36- 0.317 and 37- 0.328 (Dagnachew *et al*., 2011). Frequencies of these SNPs did

Dagnachew & Ådnøy (2014) also report and they were still higher than the other SNPs. The linkage disequilibrium (LD) in the SNPs on CSN3 was very strong (Hayes *et al*., 2006).

Haplotypes

Hayes *et al*. (2006) found eight unique haplotypes for CSN3 in Norwegian goats and they had significant effects on protein percentage. One haplotype had an increasing effect on protein percentage but also a decreasing effect on fat percentage while another haplotype had negative effects on both traits (Hayes *et al*., 2006). Dagnachew & Ådnøy (2014) did also found that the protein percentage was significantly affected of the haplotypes in CSN3 in Norwegian goats.

Material and methods

DNA- sequencing

The DNA samples were extracted from nose swabs from Performagene™ with 0.5 ml sample liquid. Nose swabs were distributed to goat farmers on the yearly meeting for the Swedish goat breeders' association in February 2018 and send by mail to other goat owners and breeders. It was the owners themselves that took the samples from their goats. One DNA sample were taken from the goat herd of SLU, in this case it was the author of this paper that was the sampler. The DNA samples used, came from goats all around Sweden and the number of the different breeds used can be seen in table 3.

Table 3. The number of DNA and milk samples from each breed

Breed	DNA samples	Milk samples
Swedish Landrace goat	26	17
Jämt goat	9	0
Swedish Landrace goat x Jämt goat	3	0
Lapp goat	6	0
Göinge goat	4	0
Total	48	17

Extraction of DNA

The extraction of DNA from the swab samples were done following the laboratory protocol for 0.5 ml of Performagene™ sample (sample liquid). The nose swab samples were shaken and incubated in 50ºC water for one hour. The sponges were pressed from the sample liquid and removed. 500 µl sample liquid were transferred to an eppendorf tube, 20 µl of PG-L2P purifier were added, vortexed and then incubated on ice for 10 minutes. The samples were centrifuged at room temperature for five minutes at 13,000 RPM and then 500 µl of the clear supernatant were transferred into a new eppendorf tube, separated from the pellet that was formed during centrifuging. The supernatant was mixed with the added amount of 25 µl of five M NaCl and 600 µl of room temperature 99.97 % ethanol were added and mixed before it stood in 10 minutes in room temperature to let the DNA to precipitate. The samples were centrifuged again at 13,000 RPM in two minutes in room temperature and the supernatant were carefully removed without disturbing the remaining pellet that contains the DNA. The DNA was washed with 250 µl of 70 % ethanol and stood for one minute in room temperature before the ethanol was removed. The samples were centrifuged to pool the remaining ethanol and dissolve it. In the tube with the washed DNA, 100 µl of DNA buffer (ATE- buffer) were added, vortexed and left during the night in room temperature. The next day was the DNA-concentration checked with a Nanodrop, ND 8000 and the belonging software: ND 8000 2.3.2.

Sequencing of DNA

The BigDye® Direct Cycle Sequencing Kit from Applied Biosystems® by Life Technologies™ was used for the sequencing. The DNA was diluted to 4 ng/µl with a total volume of 50.0 µl in each tube including DNA and deionized H_2O. The forward primer was

GAGCTTCAACAAAAGTCTTTCCA and started at intron 11 at the gene CSN1S1 and the reverse primer was TGACTTCATAGTTCAAATGCACA and ended at intron 13 on the same gene. The primers are the same as in Mestawet *et al*. (2013) and this sequence with exon 12 and 13 are according to Mestawet *et al*. (2013) and the exon 12 is also according to Hayes *et al*. (2006). According to National Center for Biotechnology Information (NCBI), exon 12 is named exon 11. The primers used had M13 tails and was diluted to 0.8 µM. A mix for the Polymerase Chain Reactions (PCR) was prepared with 1.0 µl of DNA, 1.5 µl each primer, 5.0 µl of BigDye® Direct PCR Master Mix and 2.5 µl of deionized water for each reaction. 10 µl of the mix for each reaction were pipette in each well in a plate. The PCR cycler (PCR 1) can be seen in table 4.

Table 4. The PCR cycler for the first PCR

Stage	**9700 thermal cycler**	
	Temp	**Time**
Hold	96ºC	5 min
Cycle (35 cycles)	94ºC	30 sec
	62ºC	45 sec
	68ºC	45 sec
Hold	72ºC	2 min
Hold	4ºC	∞

Sequencing were done with a mix of 2.0 µl of BigDye® Direct Sequencing Master Mix and 1.0 µl of either BigDye® Direct M13 forward or reverse primer. 3.0 µl of this mix were pipette into each reaction in the plate from the PCR 1. The cycle sequencing was then running with the cycler in table 5.

Table 5. The PCR for cycle sequencing

Stage	**9700 thermal cycler**	
	Temp	**Time**
Hold	37ºC	15 min
Hold	80ºC	2 min
Hold	96ºC	1 min
Cycle (25 cycles)	96ºC	10 sec
	50ºC	5 sec
	60ºC	4 min
Hold	4ºC	∞

After the cycle sequencing, the reactions were cleaned with a mix of 45 µl of SAM™ Solution and 10 µl of XTerminator® Solution per reaction. A volume of 55 µl were added to each

reaction and the plate were vortexed in 2500 rpm for 20 min and was centrifuged for 2 min in 1000x g. The plate was then sequenced in a capillary electrophoresis (CE), 3500xL Genetic Analyzer from Applied Biosystems®.

Analysing the sequenced data

The software Chromas version 2.6.5 from Technelysium Pty Ltd © was used for analysing the sequences from the CE. The different versions of sequences with polymorphisms of exon 12 from Hayes *et al*. (2006) were used to find the location where the deletion and the other possible polymorphisms were in the Swedish goats. The allele frequencies were calculated at the locus of the deletion, in total of all individuals and by breed. Indels in other locations of the sequenced DNA where also checked and calculated frequencies of in number of goats.

Casein and whey protein composition in the milk

Seventeen milk samples were collected from different Swedish Landrace goats, 10 individuals from herd A, four individuals from herd B, two individuals from herd C and one individual from herd D. From herd A, bulk milk was also analysed at four different occasions during the lactation. From herd E and F, the bulk milk was analysed once. To determine the protein levels, a CE system (Agilent Technologies Co., USA) was used. The samples were first de-fated in a centrifuge in 10 minutes, 3000 RPM and 4ºC. The de-fated samples were heated in a water bath in 45ºC in 15 minutes twice and vortexed in-between. In order to denaturize the casein micelle, sample buffer (SB) was prepared with Triss, EDTA, MOPS, Urea and MHEC. All chemicals were obtained from Sigma-Aldrich if not stated differently and all the concentrations of the chemicals where as followed in Johansson *et al*. (2014). Freshly made dithiothreitol (DTT) was added (0.0221g in 8.5 ml SB) before the analyses. 150 µl of milk from each sample were individually mixed with 350 µl of SB. These were vortexed and then left for reacting in one hour in room temperature. The samples were filtered through Nylon Syringe Filters with a pore size of 0.45 µm and a diameter of 13 mm into new Eppendorf tubes. From these tubes 30 µl were taken into conical tubes, designed for the CE analyses. In the machine, a run buffer with Trisodium citrate dehydrate, citric acid, urea and MHEC were used. The software used for analysing the protein levels was Chemstation. For estimation of the relative protein amount the electropherogram where analysed using the valley-to valley method. Representative example of the electropherogram is shown in figure 1.

For the statistical analysis, Microsoft excel was used to calculate and get an overall perspective of proportions of the individual caseins. The goat individuals and the bulk milk results were also divided into three classifications, low (0-6.99%), medium (7-14.99%) and strong (15-20%), according to the proportion of α_{s1}-CN from the total protein identified.

Results

DNA- sequencing

The DNA concentrations from the extraction varied between 20.9 ng/µl and 3924 ng/µl. The mean value for all the concentration measures was 451.5 ng/µl but the median value was 167.4 ng/µl. Half of all the measures were under 200 ng/µl.

Out of 48 sequenced samples, there were only 42 samples that were readable. Results were calculated from 24 Swedish Landrace goats, seven Jämt goats, three Göinge goats, six Lapp goats and two crosses between Swedish Landrace goat and Jämt goat.

Polymorphisms and allele frequencies

From the sequencing, three different genotypes at the locus for the current deletion were found; D'/D', G'/D' and A'/D' were D'/D' is the deletion. How these were located in the sequence can be seen in table 2 and calculated frequencies can be seen in table 6. Table 7 shows calculated allele frequencies separated among the different breeds.

Table 6. Genotype and allele frequencies of the different polymorphisms. The genotype frequencies are presented in both the number of the current polymorphism and the proportion. The allele frequencies are only presented in proportion and for D', G' and A' respectively in the right column

Polymorphism	**Genotype frequency**	**Allele frequency**
D'/D'	37 (88.09 %)	94.05 %
G'/D'	3 (7.14 %)	3.57 %
A'/D'	2 (4.76 %)	2.38 %

Table 7. Allele frequencies in the different breeds. Swe.L. = Swedish Landrace. In the Göinge goat, there were two D'/D' and one A'/D'

	Allele frequencies				
Allele	Swe.L. goat	Jämt goat	Göinge goat	Lapp goat	Swe.L. x Jämt
D'	91.67 %	100 %	83.33 %	100 %	100 %
G'	6.25 %	-	-	-	-
A'	2.08 %	-	16.67 %	-	-

Other mutations

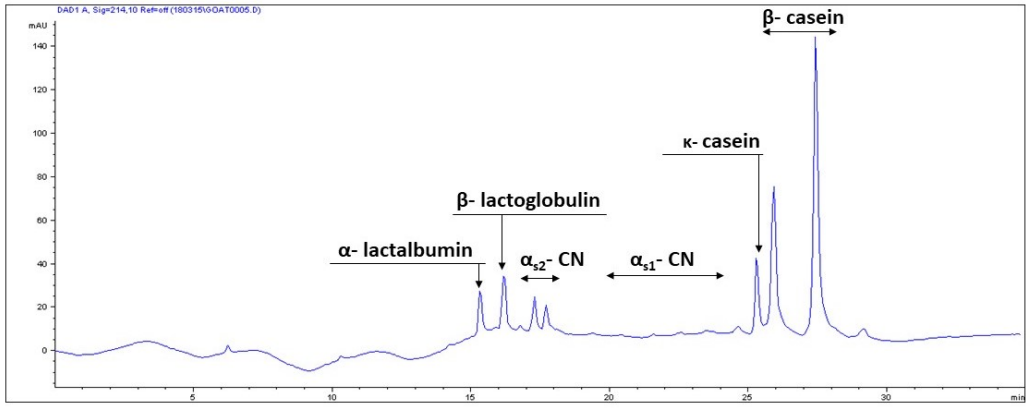
A potential difference in the sequence located close to the deletion was found in one individual. The sequence had a C instead of an A two bp after the deletion in the direction 5'-3', which can be seen in table 8. An indel was also observed at the 11th intron of heterozygote individuals, however, this was not detected in homozygous individuals. The difference is seen in table 8.

Table 8. Difference in the sequence with a C instead of an A close to the deletion in exon 12 (in the first row) and sequence difference between the homozygous and heterozygous individuals where the indel occur in the 11th intron, marked in italic (in the second row).

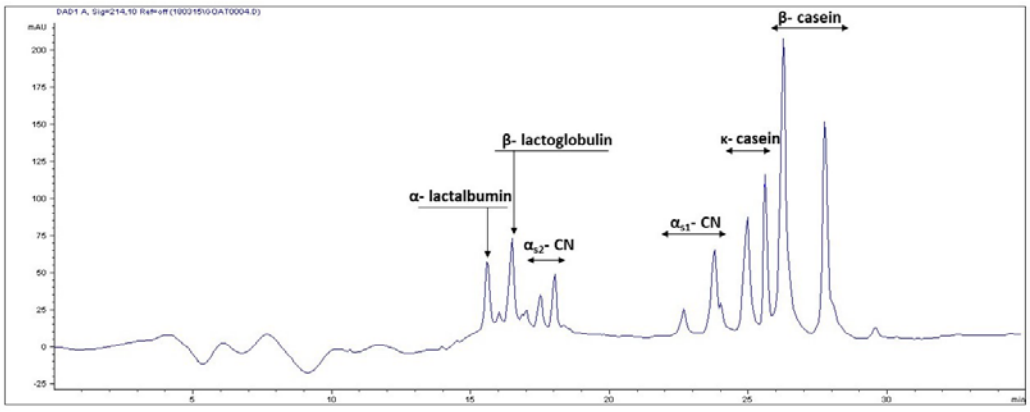
Sequence homozygous for the deletion	**Sequence with other mutation**
CTGAAAAATAC	CTGAAACATAC
GTTAAAACAATGT	GTTAAAA*MMAWKK*

Casein composition from milk

The total protein profile in the milk samples was analysed with emphases on α_{s1}- CN. An example of a protein profile with low expression of α_{s1}- CN can be seen in Figure 1a and with medium expression of α_{s1}- CN in Figure 1b. The largest fraction of total casein corresponds to β- casein and the smallest to α_{s1}-CN with κ- casein and α_{s2}- CN in between. Results on the proportions of the different caseins of all caseins in the milk for both individuals and bulk milk can be seen in Figure 2.



a.



b.

Figure 1. a. A protein profile from the software Chemstation that shows low expression of α_{S1}- CN.
b. A protein profile from the software Chemstation that shows medium expression of α_{S1}- CN.

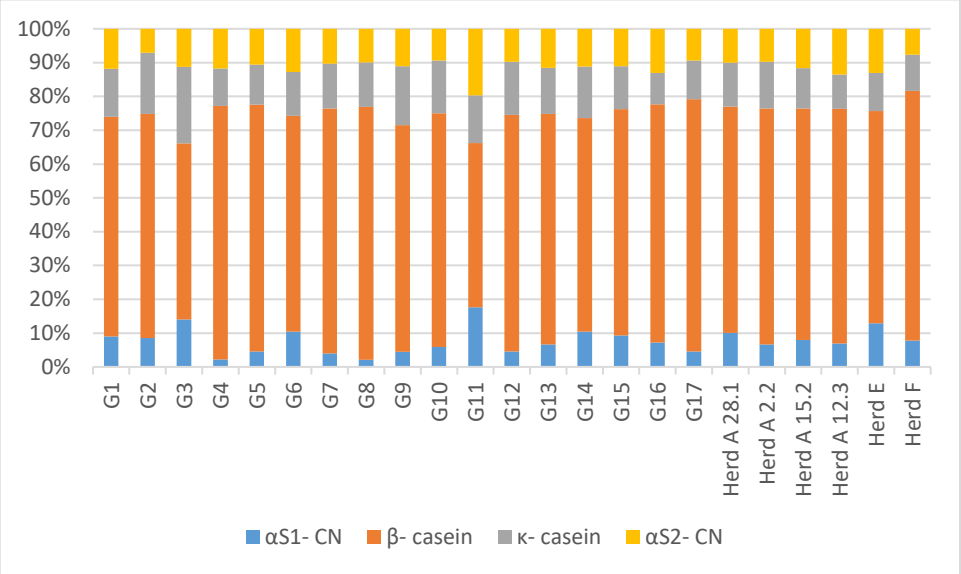


Figure 2. Proportion of α_{s1}- CN, β- casein, κ- casein and α_{s2}- CN of all caseins from different individuals and tank milk with dates. The individuals were named so G1-G10 were from herd A, G11 was from herd D, G12-G13 were from herd C, G14-G17 were from herd B. The bulk milk samples were from herd A at the different dates: 28th of January, 2nd of February, 15th of February and 12th of March in the year of 2018 and from herd E and F.

Out of the 17 individuals measuring the casein levels, nine goats (53 %) had low proportion of α_{s1}- CN, seven goats (41 %) had medium proportion and one goat (6 %) had strong proportion of α_{s1}- CN. When including the bulk milk, the numbers look different. The bulk milk from herd A with the first and the last sampling occasions had low expression but the other two occasions from herd A and bulk milk from herd E and F classified into medium expression. The proportion for the classifications of α_{s1}- CN for bulk milk where then 48% for low, 17% for medium and 4% for strong.

On the other hand, the combined sequencing and casein composition results shows that all the heterozygotes were classified with medium or strong expression of casein proportion. Five of the homozygotes were also classified as medium and nine of the homozygotes were classified as low.

Discussion

In this study, the aim has been to see if the deletion that is present in exon 12 of the gene CSN1S1 in Norwegian goats is also present in Swedish goats. In Norway, this gene, exon and deletion has been followed up during several years from the year of 2006 (Hayes *et al*., 2006; Dagnachew *et al*., 2011; Dagnachew & Ådnøy, 2014). These studies include the effects from SNPs in the casein genes and updates new frequencies and new effects of them in the Norwegian goat population. The updated frequencies show that the work of selection of goat bucks, that started the year of 2008 in Norway, with information about the genotypes of the SNP where the deletion is located, has had effect when the frequency of the deletion has decreased from 0.745 (Hayes *et al*., 2006) to 0.676 (Dagnachew & Ådnøy, 2014). In the year of 2014, the selection was based on both the SNP on exon 12 and the other SNP on exon 9 (Dagnachew & Ådnøy, 2014). Before that selection started, the selection were based on dry matter content in the milk (Hayes *et al*., 2006) while France had a selection more specific to increase the alleles of CSN1S1 that gives most casein production (Frattini *et al*., 2014). The outcoming difference in these two different breeding goals is that the breeds in France has lower frequencies of null alleles than the Norwegian breed had before 2014. The Norwegian breeding goal was not specific enough to decrease the frequency of null alleles.

Studies about the genetics of the low expression of α_{s1}- CN have not been done in Sweden before. But it has been thought that the deletion on exon 12 also exists in Swedish goats. The current study showed that this deletion does exist in the Swedish goats, and not only in the biggest breed, the Swedish Landrace goat, but as well in the smaller breeds Jämt goat, Göinge goat and Lapp goat. The deletion occurs throughout Sweden. Of the genotyped goats in this study, the frequency of the deletion was higher than in the Norwegian studies, even before the selection of Norwegian bucks on the SNPs started in 2008. This Swedish frequency cannot be confirmed properly now with only this study of 42 individuals genotyped, it needs more studies to confirm the high allele frequency of 0.941 of the deletion and have more reliable results. The high frequency can be explained by the fact that the homozygosity and heterozygozity of the deletion gives higher milk yield (Dagnachew *et al*., 2011) and that is mainly what farmers breed for. The higher milk yield could be an evolutionary benefit from natural selection because milk is naturally for kids and not for cheese production. The homozygosity of the deletion also gave lower fat, lactose and protein percentages in the milk (Dagnachew *et al*., 2011), so the milk gets more diluted. Why the frequency is higher in Swedish goats than in Norwegian could be because of the smaller population size of Swedish goats. According to the Swedish goat breeders' association (2015), the number of Swedish Landrace goats was 50,000 in the year of 1920 but only 3,000 individuals in the year of 2002. In the year 2013, there were at least 2,600 goats in this breed in Sweden. This indicates that a "bottle neck" has occurred in the breed and important alleles can have disappeared that exists in Norway where the decrease of number of goats has not been as drastic during the 20th century as in Sweden (FAOSTAT, 2017a). During this "bottle neck" the deletion could have increased in frequency in Sweden. The current study showed that all individuals that had been genotyped had the deletion allele even though five of them were heterozygote for it with the alleles G' and A', the same alleles that Hayes *et al*. (2006), Dagnachew *et al*. (2011) and Dagnachew & Ådnøy (2014) reported were the other possible alleles for the SNP. But even for this, further studies are required for a better result of the allele frequencies and if it exists individuals of goats in Sweden that does not have the D' allele. From the results of this study it seems like the breeds Jämt goat, Lapp goat and the crossbreed only had the D' allele and

that Göinge goat only had the D' and A' alleles. But this is far from reliable because of the few number of individuals in these breeds. An increased number of individuals in these breeds needs to be genotyped to make a conclusion if there actual is differences in the alleles between the Swedish breeds.

Due to that the deletion in exon 12 exists and was in high frequency in the Swedish goats, this needs to take notice of and be remembered in the breeding work of these goats. If this high frequency is true, the deletion on this SNP could soon be a fixed homozygosity with the consequence of low expression of α_{s1}- CN. With higher expression of α_{s1}- CN, the cheese yield can be higher with the same amount of milk. But it is important not to select only the individual goats that does not carry the deletion or is heterozygote for it in the breeding, it needs to be some homozygote individuals for breeding. Otherwise, there is a high risk for high inbreeding.

Those individuals that were heterozygote for the deletion had also an indel in intron 11 that the individuals homozygote for the deletion did not had. This indel could be high in LD with the locus with the deletion on exon 12 and is interesting to continue to study. If more individual goats in Sweden heterozygote for the deletion also have the indel and if it in this case is a qualification to have the indel to be heterozygote at this SNP. Another mutation found is only in one individual and it was a different allele close the SNP with the studied deletion. The analysis showed both the deletion and a C instead of an A two bp from the SNP. This could be a new mutation, but it is not likely because the C peak was not clear and it was only in one individual it occurred. It is more likely that it was a mistake in the sequencing. Several sequences and reads were not readable which lead to the fewer amounts of samples analysed and some reads were blurry and hard to read. Some samples only had one of two reads readable which lowered the certainty in the sequencing results. The failed and blurry sequences and reads should have been redone to make certain of their genotype.

The sequences worked for inter alia the individual goats that also had milk samples and these both results could be merged. The merged result showed that no heterozygote individual expressed low proportion of α_{s1}- CN even though Dagnachew *et al*. (2011) reported that heterozygosity with G'/D' gave similar effects as homozygosity for the deletion. That can explain why some homozygotes could be classified into medium expression. But why some homozygotes express more α_{s1}- CN than others can be because of some SNP where they had another allele or environmental factors. Unfortunately, there were no milk samples from the goats that had the genotype A'/D'. If there were, the question is then if it would be a difference in the expression of α_{s1}- CN from the genotype G'/D'? The literature says it can be (Dagnachew *et al*., 2011), but it is not tested in Sweden. A theoretically question is also if the α_{s1}- CN expression will increase to the classification strong for an individual goat that does not carry the deletion on exon 12.

In the literature, different papers present different proportions of α_{s1}- CN and the other caseins of the total of caseins. The different numbers are from different breeds and no one of them is from Scandinavia so that makes it hard to compare the proportions with the results in this study. Johansson *et al*. (2014) reported proportions of the caseins in Swedish goats but these probably carry the deletion, at least many of them due to the sequencing results in this study. The allele frequency of this deletion could be high and defines the breed specific proportions of caseins. But the breed specific proportions of the caseins could also be represented from

individuals without the deletion in Swedish Landrace goat, Jämt goat, Lapp goat and Göinge goat.

In this study, both milk from individual goats and bulk milk were analysed. From herd A, the individual milk samples varied in α_{s1}- CN proportion but the bulk milk from the same herd were more stable. So even if some individuals had low proportion of α_{s1}- CN, the other individuals with higher proportion compensate for the low proportions in the bulk milk.

Dagnachew *et al*. (2011) and Dagnachew & Ådnøy (2014) reported about the other mutation in CSN1S1, a deletion in exon 9 that together with the deletion in exon 12 caused the low expression of α_{s1}- CN in the milk from Norwegian goats. This SNP in exon 9 has not been analysed in Swedish goats in the current study. Due to that the deletion in exon 12 did exist and had a high frequency in Swedish goats, the deletion in exon 9 is probably also present in the Swedish goats and affect the expression of α_{S1}- CN in the milk. This SNP is worth to study in Swedish goats due to that this could cause the variation of low and medium expressions in the individual goats in this study that all are homozygote to the deletion in exon 12.

Conclusion

This study aimed to observe the exon 12 in the gene CSN1S1 in Swedish goats to see if the deletion in this exon that been found in Norwegian goats also exist in Sweden and if there were any differences between the Swedish breeds. In conclusion, this deletion does exist in all the breeds Swedish Landrace goats, Jämt goats, Lapp goats and Göinge goats. About the differences in DNA between the breeds, it is too few individuals analysed to make a conclusion. It needs further studies with more individuals to make a reliable conclusion about the allele frequencies in the Swedish goats, if the deletion exists in every individual and if there are any differences between the breeds. Anyway, this deletion detected in exon 12 provides insight to consider in the breeding programs. However, extensive analysis of the gene with representative samples id highly required to arrive concrete conclusion. But it is important not to select to strongly in the breeding program due to that inbreeding will quickly be a problem when only selecting on heterozygotes when it seems to occur many homozygotes for the deletion in this population. Exon 9 could also be considered for further study together with exon 12. Significant contribution of the former exon region was revealed in Norwegian goats.

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