# CSN1S1 Gene Polymorphism of Indonesian Local PE, Saanen, and Sapera Goats

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**ABSTRAK**

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CSN1S1 (alpha-s1 casein) gen merupakan salah satu dari empat gen utama pengontrol produksi kasein susu. Variasi genetik pada gen ini diketahui mempengaruhi kualitas protein susu kambing perah. Studi ini mempelajari polimorfisme genetik dari gen CSN1S1 menggunakan metoda *direct sequencing* pada kambing lokal Peranakan Etawah (PE), eksotis Saanen, dan Sapera sebagai kambing persilangan keduanya. Sampel darah dikumpulkan dari induk betina pada kambing Sapera 66 ekor, kambing Saanen 15 ekor, dan kambing PE 14 ekor dari Balai Penelitian Ternak di Bogor, Jawa Barat. Program MEGA11 digunakan untuk menganalisis data sekuen gen CSN1S1 pada fragmen DNA ekson 12 dan parsial intron 12. Paket program Popgen 3.2 dipakai untuk menganalisis frekuensi alel dan genotipe, serta nilai keseimbangan Hardy-Weinberg (H-W), dan tingkat heterosigositas (Ho). Teridenditikasi ada empat SNP: g.10243 G>A, g.10250 A>G, g.10277 G>A, dan g.10283 T>G. Berdasarkan frekuensi alel dan genotipe, nilai keseimbangan H-W dan tingkat Ho menunjukkan bahwa gen CSN1S1 pada keempat SNP tersebut bersifat polimorfik untuk ketiga genotipe kambing. SNP teridentifikasi dapat dipertimbangkan sebagai kandidat penanda seleksi kualitas protein susu. Penelitian ini memberikan wawasan tentang keragaman genetik gen CSN1S1, sebagai dasar seleksi molekuler untuk meningkatkan kualitas protein susu pada PE lokal dan persilanganny.

**Kata Kunci**: Gen CSN1S1, Kambing Perah , SNP

**ABSTRACT**

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CSN1S1, or alpha-s1 casein, gene is one of milk's four significant casein-controlling genes. Genetic variations in this gene have been proposed to affect milk protein quality in dairy goats. This study investigated the genetic polymorphism of the CSN1S1 gene using a direct sequencing method in local Peranakan Etawah (PE), exotic Saanen, and their cross or Sapera goats. Blood samples were collected from 66 Sapera, 15 Saanen, and 14 PE does at the Indonesian Research Institute for Animal Production (IRIAP) in Bogor, West Java Province. The MEGA11 program was used to analyze the sequence data of the CSN1S1 gene in DNA fragment exon 12 and partial intron 12. A Popgen 3.2 packet program was applied to analyze allele and genotype frequencies, Hardy-Weiberg (H-W) equilibrium, and the degree of heterozygosity (Ho) values. Four SNPs were identified: g.10243 G>A, g.10250 A>G, g.10277 G>A, and g.10283 T>G. Based on the allele and genotype frequencies, H-W equilibrium, and Ho values, the CSN1S1 gene at the four loci was polymorphic in all three goat genotypes. These SNPs may be considered candidate selection markers for milk protein quality. This study provides insights into the genetic diversity of CSN1S1, which is crucial for molecular selection to improve milk protein quality in local PE and its crosses.

**Key Words**: CSN1S1 Gene, Dairy Goat, SNP

## INTRODUCTION

The Indonesian community currently consumes approximately 4.3 million tons of milk; however, only 22% of this amount comes from domestic production, primarily cow milk. Although cow milk production is significantly higher, dairy goat milk presents a viable development opportunity owing to lower production costs and consumer benefits (Hammam et al. 2022). As the market trend for goat milk consumption grows, production has significantly increased worldwide. Compared to cow milk, goat milk has more important nutrients, including protein, fat, calcium, phosphorus, iron, vitamin A, and several B vitamins. However, it contains less lactose, which is advantageous for those intolerant to lactose (Nayik et al. 2022). The chemical structure, protein structure, and amino acid composition of goat milk decrease the probability of allergies. Furthermore, it is easier to digest, especially in patients with gastrointestinal disorders, because of its smaller fat

globules and higher levels of short- and medium-chain fatty acids (Stergiadis et al. 2019).

Genetic and breeding practices substantially influence animal milk production's quantitative and qualitative aspects. Higher milk production can be achieved by selecting specific breeds or individuals within breeds, leading to a significant increase in the milk yield over time (Brito et al. 2021). Certain breeds or individuals may also produce milk with high protein, fat, or specific minerals and vitamins (Zaalberg et al. 2019; Rahmatalla et al. 2021). Casein is the main protein in milk and accounts for as much as 80-85% of the total protein content (Chauhan et al. 2021; Rahmatalla et al. 2022). It is essential to absorb calcium microelements in newborn digestion (Rezaei et al. 2016). Casein proteins constitute a significant portion of the total protein content of milk. These are essential for forming micelles, which are crucial for transporting calcium, phosphate, and other minerals (Chauhan et al. 2021; Hammam et al. 2022; Nayik et al. 2022). From an industrial perspective, high-casein milk could yield a greater quantity of cheese curd (Wang et al. 2019). CSN1S1 (alpha-s1 casein) gene is one of the four genes responsible for milk's casein content. CSN1S1 gene plays an important role in casein secretion (Hassanin et al. 2022). Research has shown low beta-casein and kappa-casein transportation in low-expressed alpha-s1 casein in goat milk. Approximately 40% of cow milk contains alpha-S1 caseins, whereas goat milk has varying levels of casein expression owing to highly polymorphic casein genes. The CSN1S1 gene is composed of 19 exons and 18 introns (Hassanin et al. 2022; Rahmatalla et al. 2022). Base mutations (SNPs) and their interactions within the same or various genes might cause variable genetic responses in different breeds of the same animal (Dettori et al. 2024). Some previous studies have looked at casein gene genetic variants and how they affect milk components in *goat* breeds. High genetic variability in the CSN1S1 gene and the significant effects of its SNPs on the concentration of milk components have been discovered by previous studies on the CSN1S1 gene in goats (Anggraeni et al. 2021; Nayik et al. 2022; Khaldi et al. 2023).

The Peranakan Etawah (PE) goat is the only Indonesian local goat that can be used for meat and milk production. Breeding programs to improve the genetics of milk production and quality of this goat have not been extensively implemented; consequently, milk production and quality remain suboptimal (Anggraeni et al. 2020). In contrast, the Saanen dairy goat breed is the most-reared dairy goats worldwide because of their high milk yield (Devendra & Hannein 2019). However, these dairy goats are native to subtropical areas and are not adaptive in tropical regions. The development of Sapera, an improved dairy goat breed, resulted from a breeding program conducted by researchers at the IRIAP. This innovative cross-mating, comprising an equal genetic contribution from Saanen and PE goats, emerged from early scientific investigations (Anggraeni et al. 2020). This breeding program aims to produce high milk and protein yields. A preliminary study on genetic polymorphism of the CSN1S1 gene at the g.12164G>A locus showed high genetic variation with the frequency of the G allele against the A allele higher in PE goats (0.578) and Saanen goats (0.625) but lower in Sapera goats (0.333) (Anggraeni et al. 2021).

Thus, this study aimed to increase our understanding of the genetic polymorphisms of CSN1S1 using sequencing techniques in local PE, Saanen, and Sapera goats. Understanding the genetic diversity of CSN1S1 is crucial for developing effective breeding programs that aim to enhance the milk protein quality of local PE dairy goats and their crosses.

## MATERIALS AND METHODS

### Location and period of research

The Dairy Goat Research Station, a division of the Indonesian Research Institute for Animal Production (IRIAP), Ciawi, Bogor, West Java, Indonesia, conducted research on animal observations and the collection of milk and blood from dairy goats in 2021 and 2022. The IRIAP is in Banjarwaru Village within the Ciawi Subdistrict of Bogor Regency, West Java, Indonesia. IRIAP is located at an altitude of over 350 m asl with an average temperature of around 25.2$^{o}$C (22.8-33.2 $^{o}$C) and an average relative humidity (RH) of 87 5 % (73-94 %).

Molecular analysis of the CSN1S1 gene was performed at the Laboratory of Animal Molecular Genetics, part of the Department of Animal Production and Technology, at the Faculty of Animal Science of Bogor Agricultural University.

### Feeding management

All animals were maintained optimally and fed regarding physiological status. Green fodders (leaves and grass) were fed approximately 10% of body weight (ad libitum), around 3-4 kg/hd/d. Meanwhile, concentrate feed was given as a source of protein and energy with a protein content of between 16-18% with TDN 70-80%, about 2% of body weight or 0.2-1 kg/hd/d. Concentrate is given to the does around 0.8-1 kg/hd/d.

### Research sample

Ninety-five blood samples were analyzed at the Animal Molecular Genetics Laboratory of the Faculty of Animal Science of Bogor Agricultural University. Blood samples obtained were from all the does of the three goat genotypes, including the local PE breed (n= 14 hd.), exotic Saanen breed (n= 15 hd.), and cross Sapera genotype (n= 66 hd.), whose blood composition is 50% PE and 50% Saanen.

These goats were in various stages of lactation, ranging from 1 to 8 months, with periods of lactation from 1 to 4 and a kidding period from 2021 to 2022. All animals were maintained at the dairy goat experimental station of the IRIAP, Ciawi sub-district, Bogor, West Java, Indonesia.

Fresh blood (approximately 3 ml per sample) was collected from each animal's jugular vein. The samples were transferred to a 10-ml EDTA-containing tube and stored under refrigeration for preservation and subsequent analysis.

**Molecular analysis**

DNA extraction was performed at 10-50 µg/mL. PCR was performed on an AB system machine. Primers were created manually and optimized using Primer Stat software. The primers used for the amplification of exon 12 and a partial segment of intron 12 were designed with the forward primer (F) sequence for 5'-CTCATCCTCTGTCCTCTTCT -3' and the reverse primer (R) sequence for 5'-CTGTGCTTTCACAAGGAGGC -3' (Figure 1).

The PCR process was performed in a 26 µL reaction mixture containing 2 µL (10-50 µg/mL) DNA extract, 0.3 µL (10 µM) forward primer, 0.3 µL (10 µM) reverse primer, 10.4 µL distilled water, 12.5 µL My Taq Hs red mix produced by BioScience (USA), and 0.5 µL MgCl2. The temperature profile was 1 minutes for initial denaturation at 95°C, 35 cycles of 15 s at 95°C, 30 s at 60°C, and 10 s at 72°C, with a 5 min final extension at 72°C. Sanger sequencing was used to analyze the DNA sequences of exon 12 and partial intron 12 of CSN1S1. Direct sequencing using Sanger sequencing was conducted at the Macrogen Laboratory in South Korea to analyze the sequence of the targeted amplicon.

**Data analysis**

Hardy-Weinberg (H-W) equilibrium and the degree of heterozygosity were assessed using the equation following the study by Anggraeni et al. (2021) to calculate allele and genotype frequencies.

**Ethical approval**

The methodology employed in this study was approved by the ICARD Experimental Animal Welfare Commission of the Indonesian Agricultural Research and Development Agency (approval number ICARD/IRIAP/Rm/11/2021).

## RESULTS AND DISCUSSION

**CSN1S1 gene sequence**

In our study, utilizing AJ504710.2 as the reference base, four SNPs were detected in the CSN1S1 fragment target in all goats. The SNPs were identified for g.10243 G>A, g.10250 A>G, g.10277 G>A, and g.10283 T>G (Figure 2, Figure 3). The numerical designations represent the specific positions within the CSN1S1 gene sequence. For instance, SNP g.10243 G>A indicates a substitution from Guanine (G) to Adenine (A) at the 10243$^{rd}$ base pair from the initiation of the CSN1S1 gene. Two of these SNPs, g.10243 G>A and g.10250 A>G, were identified in the exon 12 region of the CSN1S1 gene. The exon region of a gene is responsible for encoding proteins; consequently, mutations in this region may affect the structure and function of the protein encoded by the CSN1S1 gene (Lim et al. 2018).

The CSN1S1 gene is involved in the production of casein, which affects milk quality (Rahmatalla et al. 2021; Rahmatalla et al. 2022). Hence, these SNPs might have significant implications for milk protein. The CSN1S1 gene is known to be involved in the production of casein, which affects milk quality (Hassanin et al. 2022). Consequently, these SNPs may have significant implications for milk protein composition. The presence of two SNPs in the gene's coding region suggests a potential impact on the structure of the casein protein.

These findings open up new possibilities for studying the genetics of milk production and quality in goats, particularly the potential for breeding goats for higher-quality milk based on their CSN1S1 gene sequence. This investigation identified a missense mutation in caprine subjects' exon 12 region of the CSN1S1 gene. Specifically, the single nucleotide polymorphism (SNP) g.10243 G>A resulted in an amino acid substitution from Arginine (amino acid code: R) to Lysine (amino acid code: K). This alteration could affect the milk protein quality and functionality. Amino acids play a critical role in protein structure and function.

Arginine and Lysine are essential amino acids with distinct properties (Meuzelaar et al. 2016). Arginine contains a guanidin group, which can form multiple hydrogen bonds, whereas Lysine features an amino group in its side chain (Rahmatalla et al. 2021). These two amino acids share similarities in their overall charge. The variations in their side chains may influence protein folding, stability, and function (Meuzelaar et al. 2016). Missense mutations in the CSN1S1 gene have been demonstrated to influence dairy goats' milk protein composition, yield, and quality (Widodo et al. 2023). A mutation involving the substitution of Arginine with histidine has been observed to correlate with a diminished presence of αs1-casein in milk, subsequently influencing coagulation characteristics and cheese production efficiency (Hammam et al. 2022). The findings indicate that alterations in amino acid sequences can impact milk quality, although the specific mutation observed in the referenced study differs from the one examined in our research. Nevertheless, the specific impact of the Arg-to-Lys mutation in the CSN1S1 gene on milk protein quality and function remains insufficiently examined.


**Figure 1**. Visualization of forward and reverse primers. The reference base sequence is AJ504710.2


**Figure 2**. Visualization of mutation points occurring in the target amplicon. SP denotes the Sapera goat, PE denotes the PE goat, and SA denotes the Saanen goat. The reference base was obtained from AJ504710.2

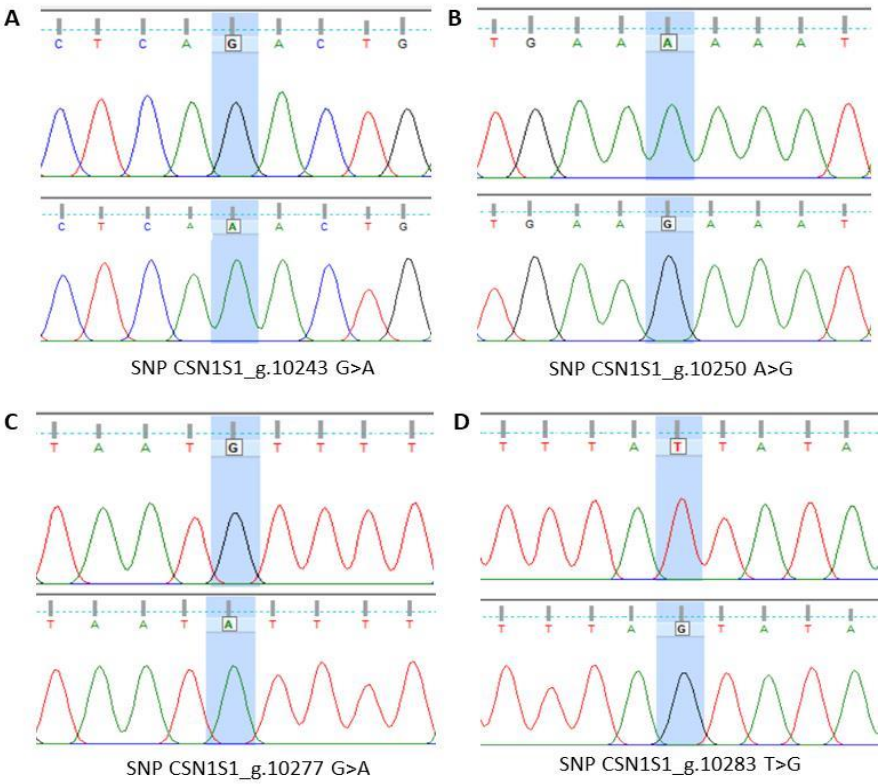


**Figure 3**. The sequence chromatograms of the SNPs from the CSN1S1 gene exon 12 and partial intron 12 (obtained from Finch TV images). The upper and lower panels depict non-mutant and mutant genotypes, respectively. (A) SNP CSN1S1_g.10243 G>A (B) SNP CSN1S1_g.10250 A>G (C) SNP CSN1S1 g_10277 G>A (D) SNP CSN1S1_g.10283 T>G

**Genotype and allele frequencies**

Table 1 presents the genotyping results of the SNPs present in the targeted amplicon from Sapera, Saanen, and PE goats. Across all four SNP locations (CSN1S1_g.10243 G>A, CSN1S1_g.10250 A>G, CSN1S1_g.10277 G>A, and CSN1S1_g.10283 T>G), a predominantly consistent genotype is observed in all three genotypes.

Moreover, the allele frequencies exhibit minimal variation across the breeds for each SNP. The findings, however, differed from the previous study by Anggraeni et al. (2021) concerning the genetic polymorphism of the CSN1S1 gene at the g.12164G>A locus.

The previous research found a significant genetic variation, with the G allele frequency surpassing that of the A allele in PE goats (0.578) and Saanen goats (0.625). The differences may be attributed to variations in single nucleotide polymorphisms (SNPs) within the same caprine population. A subsequent investigation conducted by (Rahmatalla et al. 2021) on eight native and exotic goat breeds identified a novel single nucleotide polymorphism (SNP) of SCN1S1 in exon 12 at locus g.7213522443A/G. This polymorphism exhibited high genetic variation in six breeds, with the A/G allele resulting in a genotype frequency ranging from approximately 0.50 to 0.75.

**Heterozygosity and Hardy-Weinberg Equilibrium**

This investigation assessed genetic diversity among the Saanen, PE, and Sapera goats, utilizing heterozygosity as a primary measure. Heterozygosity values approaching 0 indicate limited genetic diversity, whereas values nearing 1 suggest substantial genetic diversity (Sahoo et al. 2023). The observed heterozygosity (Ho) and expected heterozygosity (He) values derived from this investigation are presented in Table 2. Interestingly, none of the populations studied were in Hardy-Weinberg equilibrium (X^2 test > X^2 table). The preliminary study on the CSN1S1 gene at g. 12164G>A locus reported the SNP in H-W equilibrium for Sapera, PE, and Saanen goats (Anggraeni et al. 2021). The observation above suggests that, in the absence of external factors, genetic variation would remain constant across successive generations. Any deviation from this equilibrium state could be attributed to phenomena such as genetic drift, inbreeding, or non-random mating practices within these goat populations, which can significantly alter gene frequencies.

**Table 1**. Genotype and allele frequencies of the four single nucleotide polymorphisms (SNPs) in Sapera, Saanen, and PE goat populations

| Dairy goat type | N | Genotype | | | Allele | |
|---|---|---|---|---|---|---|
| CSN1S1_g.10243 G>A | | AA | AG | GG | A | G |
| SAPERA | 66 | 0.196 | 0.000 | 0.803 | 0.197 | 0.803 |
| SAANEN | 15 | 0.067 | 0.000 | 0.933 | 0.067 | 0.933 |
| PE | 14 | 0.071 | 0.000 | 0.929 | 0.071 | 0.929 |
| CSN1S1_g.10250 | | AA | AG | GG | A | G |
| SAPERA | 66 | 0.818 | 0.000 | 0.812 | 0.818 | 0.182 |
| SAANEN | 15 | 0.933 | 0.000 | 0.067 | 0.933 | 0.067 |
| PE | 14 | 0.929 | 0.000 | 0.071 | 0.929 | 0.071 |
| CSN1S1_g.10277 G>A | | AA | AG | GG | A | G |
| SAPERA | 66 | 0.182 | 0.000 | 0.818 | 0.182 | 0.818 |
| SAANEN | 15 | 0.067 | 0.000 | 0.933 | 0.067 | 0.933 |
| PE | 14 | 0.071 | 0.000 | 0.929 | 0.071 | 0.929 |
| CSN1S1_g.10283 T>G | | GG | GT | TT | G | T |
| SAPERA | 66 | 0.197 | 0.000 | 0.803 | 0.197 | 0.803 |
| SAANEN | 15 | 0.067 | 0.000 | 0.933 | 0.067 | 0.933 |
| PE | 14 | 0.071 | 0.000 | 0.929 | 0.071 | 0.929 |

**Table 2**. Observed heterozygosity (Ho), expected heterozygosity (He), and chi-square ($\chi^2$) test values

| Dairy goat type | N | Ho value | He value | X^2 test |
|---|---|---|---|---|
| CSN1S1_g.10243 G>A | | | | |
| Sapera | 66 | 0.00 | 0.32 | 68.24 |
| Saanen | 15 | 0.00 | 0.13 | 29.04 |
| PE | 14 | 0.00 | 0.14 | 27.04 |
| CSN1S1_g.10250 A>G | | | | |
| Sapera | 66 | 0.00 | 0.30 | 68.46 |
| Saanen | 15 | 0.00 | 0.13 | 29.04 |
| PE | 14 | 0.00 | 0.14 | 27.04 |
| CSN1S1_g.10277 G>A | | | | |
| Sapera | 66 | 0.00 | 0.30 | 68.46 |
| Saanen | 15 | 0.00 | 0.13 | 29.04 |
| PE | 14 | 0.00 | 0.14 | 27.04 |
| CSN1S1_g.10283 T>G | | | | |
| Sapera | 66 | 0.00 | 0.32 | 68.24 |
| Saanen | 15 | 0.00 | 0.13 | 29.04 |
| PE | 14 | 0.00 | 0.14 | 27.04 |

Table 2 presents the observed heterozygosity (Ho), expected heterozygosity (He), and X^2 test values for each genotype at the identified SNPs. A comparison of the actual genotype frequencies with those expected under Hardy-Weinberg equilibrium revealed significant deviations for all SNPs. This observation strongly indicates the influence of genetic forces, potentially including non-random mating, genetic drift, or selection, resulting in an observed departure from equilibrium. Elucidating the nature of these deviations is crucial for future research, particularly regarding their potential implications in population genetics and the development of effective breeding strategies (Baneh et al. 2020; Sahoo et al. 2023).

While these findings provide valuable insights into the genetic diversity of these goat breeds, it is imperative to acknowledge the limitations of our study. The sample size was relatively small, and there exists potential for selection bias. To address potential issues with statistical analysis, we implemented rigorous statistical tests appropriate for smaller sample sizes. However, these limitations do not diminish the significance of our findings but rather elucidate areas for further investigation.

Subsequent investigations employing larger sample sizes and more comprehensive analyses are necessary to corroborate and extend our findings. Such research would not only validate our results but also provide a more detailed understanding of the genetic diversity of these caprine populations. This knowledge is crucial for designing effective breeding strategies and improving the quality of *caprine* milk, a valuable resource in the dairy industry.

## CONCLUSION

Genetic polymorphism study in the CSN1S1 gene, specifically at exon 12 and partial intron 12 regions, across local, exotic, and cross-bred goats identified four single nucleotide polymorphisms (SNPs): g.10243 G>A, g.10250 A>G, g.10277 G>A, and g.10283 T>G. Notably, the g.10243 G>A SNP led to an amino acid change from Arginine to Lysine. This alteration in exon 12 of the CSN1S1 gene could potentially impact the quality of milk and protein. These discoveries offer a valuable foundation for upcoming studies for improving dairy goat milk quality through genetic selection. The outcomes further underscore the importance of understanding the genetic diversity and composition of dairy goat populations, which can be instrumental in developing effective breeding programs.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this published article, neither the funding nor the content

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