



The Relationship of POU1F1-Hinfl Gene Polymorphisms on Milk Yields in Simmental Cattle

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Abstract: As the global population grows, there is a need to produce higher yields in food, agriculture, and livestock. To achieve this, scientists are exploring new techniques and methods. However, it is crucial to select the right candidate genes and markers, especially in techniques like QTL and MAS in livestock, to ensure success. We conducted a study to determine allele frequencies and their association with milk yield in 70 Simmental cattle breeds in two lactations using the PCR-RFLP technique. The statistical analysis was conducted using the general linear model procedure with the least square method. The study focused on the allele frequencies for the POU1F1-*Hinfl* gene in Simmental cattle. The dominant B allele frequency for POU1F1/*Hinfl* was 0.58, while the A allele frequency was 0.42. Simmental population was under HardyWeinberg Equilibrium (HWE) for the POU1F1-*Hinfl* genotypes ($p > 0.05$). The study found that genotype frequencies were in balance for POU1F1/*Hinfl*. No significant correlation between POU1F1-*Hinfl* gene polymorphisms and milk yield was found, but they have been associated with growth and reproductive traits in various cattle breeds. The results could provide useful information for breeding programs aimed at improving the performance traits of Simmental cattle.

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1. Introduction

The determination of the best genotypes is hampered by the fact that the majority of economic features are influenced by polygenes and vulnerable to environmental fluctuations. In order to understand how the characteristics of the genes involved in a population are changed by non-genetic factors that might have an impact on a metric trait, statistical approaches are required. Transcription of the genes encoding the growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone (TSH) subunits is dependent on the pituitary-specific transcription factor (POU1F1; also known as Pit-1), (Li et al. 1990). These genes are involved with a wide variety of signaling pathways that are essential for a variety of physiological and developmental processes, including pituitary gland development (Li et al., 1990; Mullis 2007), mammary gland growth (Sjaunja and Olsson, 2005), expression of milk proteins, milk production, and secretion (Sjaunja and Olsson, 2005; Akers, 2006). A *Hinfl* polymorphism is located within exon 6 and characterized by a silent mutation on *Hinfl* (-) allele (Dierkes et al., 1998), and their effects were associated with growth traits in cattle (Silva et al., 2006). The available sources provide references to multiple studies that have explored the associations between POU1F1 gene

polymorphisms and different traits in cattle. POU1F1 was discovered to be associated with milk yield, protein yield, protein percentage (Zhang et al., 2009; Özdemir, 2012), fat milk production (De Mattos et al., 2004), growth traits (Kai et al., 2006; Carrijo et al., 2008) body weight (Renaville et al., 1997), some feeding criteria, and carcass dimensions in cattle (Oprzadek et al., 2003; Ozdemir et al., 2018). It was also found to be significantly associated with variation in fertilization and embryonic survival rates (Khatib et al., 2009; Laporta et al., 2011).

The marker-assisted selection techniques (MAS), applied depending on the quantitative trait locus maps created depending on the proximity of the markers to the relevant gene region or quantitative features on the chromosomes, allow the application of molecular techniques in plant and animal breeding and enable to obtain quality products with the desired characteristics and yield in a short time. Today, thanks to the developing technological methods and applications, genome-level mapping, and genome-wide association studies can be performed in breeding studies (Das et al., 2021; Ozdemir, 2021; Reshma and Das, 2021). The allele frequencies of the studies on POU1F1/HinfI gene polymorphism on different breeds between 2012 and 2023, the breeds in which the studies were conducted, and some of their numbers are given in Table 1.

Table 1. Between 2012 and 2023, several studies have been conducted to investigate the frequency of the POU1F1/HinfI gene variant

Breeds	N	Allele frequencies		References
		A (A)	B (G)	
POU1F1/HinfI				
Holstein calves	100	0.34*	0.66	(Findık and Özdemir, 2022)
Anatolian Black cattle	104	0.36*	0.64	(Sakar and Zülkadir, 2022)
Turkish Grey Steppe	137	0.46*	0.54	(Cobanoglu and Ardıclı, 2022)
Anatolian Black	105	0.91	0.09	(Cobanoglu and Ardıclı, 2022)
East Anatolian	125	0.45*	0.55	(Cobanoglu and Ardıclı, 2022)
Holstein Friesian,	50	0.32*	0.68	(Aytekin and Bayraktar, 2022)
Brown Swiss	50	0.10*	0.90	(Aytekin and Bayraktar, 2022)
Simmental	50	0.23*	0.77	(Aytekin and Bayraktar, 2022)
Iranian Holstein	134	0.32*	0.68	(Sadeghi et al., 2022)
Buffaloes	60	0.1*	0.9	(Zghairand and Hassooni, 2021)
Limousine	115	0.29*	0.71	(Pytlewski et al., 2022)
Holstein	147	0.25*	0.75	(Cañizares Martínez et al., 2021)
Polish Holstein Fresian (PHF)	1024	0.20*	0.80	(Pytlewski et al., 2018)
Holsteins	245	0.80	0.20	(Ozdemir et al., 2018)
Grati-Ongole Grade cattle	107	0.01*	0.99	(Hartati et al., 2018)
Slovak Simmental	288	0.23*	0.77	(Trakovická et al., 2015)
Holstein	100	0.25*	0.75	(Mohammadabadi et al., 2015)
Sahiwal breedcattle	77	0.19*	0.81	(Chauhan et al., 2015)
Slovak Spotted	110	0.30*	0.70	(Moravčíková et al., 2013)
Holstein	100	0.53	0.47	(Heidari et al., 2012)

*Allele gene frequencies similar to our study.

Understanding the associations between POU1F1 gene polymorphisms and traits in cattle can have implications for breeding programs. By identifying specific genetic variants associated with desirable traits, breeders can make informed decisions to select animals with favorable genetic profiles for breeding purposes.

In this study, POU1F1/HinfI gene polymorphisms were identified in the Simmental cattle breed, and their association with milk yield of genotypes and their usability in animal breeding were investigated.

2. Material and Methods

2.1. Material

In a study blood samples were collected from 70 Simmental cattle breeds, which were reared under intensive conditions in a private farm in Erzurum, from the tail vein under veterinary control and

the samples were collected in vacutainer blood tubes containing K3EDTA anticoagulant. DNA samples were extracted from the collected blood using the QIAGEN Genomic DNA Purification kit (Gentra Puregene, USA), following the manufacturer's instructions.

2.2. Methods

The primer sequences used in the PCR process was shown in Table 2.

Table 2. POU1F1/*HinfI* gene PCR primers

Gene region	Reference	Primer sequences	PCR product
POU1F1/ <i>HinfI</i>	Ozdemir (2012)	F:5-ACTCGCTATTACACAATAGGAGCCT-3 R: 5-TCCTGCCAACTCCTCACCTCCC-3	260 bp

The PCR reaction mixture (10 mL) contained; 2 µL genomic DNA, 0.25 µL F-Primer, 0.25 µL R-Primer, 2.5 µL of dNTPmix (D7595: Sigma, St. Louis, MO, USA, 0.25mM), 0.5 units of Taq DNA polymerase (D1806: Sigma), 5 µL of 10x PCR Buffer, 1.5 µL of 0.25 mM MgCl₂ and ddH₂O making the total volume of 30 µL were used for PCR amplification. The reaction conditions used in amplifications are given in Table 3.

Table 3. POU1F1/*HinfI* gene PCR conditions

Gene polymorphism	Initial denaturation	Denaturation Annealing	Extension	Number of cycles	Final extension
POU1F1	95 C/30 sec	61 C/45 sec	72 C/2 min	33	72 C/5 min

The POU1F1/*HinfI* gene was amplified using PCR, and the resulting PCR products were treated with 2-5 U of restriction enzymes and incubated at 37 °C for 12 hours. The digested products were separated on a 3.0% agarose gel for 2.0 hours at 50 Volts and visualized under UV light. The allele frequency of the base mutation of each gene was determined using POPGENE software (Francis et al., 1999) to check for Hardy-Weinberg equilibrium.

In order to investigate the influence of POU1F1/*HinfI* gene polymorphisms on lactation and milk yield traits in Simmental cattle, a variance analysis was conducted between the polymorphic regions and milk yield records of animals in different lactations. The study was carried out on a private enterprise in Erzurum, where milk production records were systematically kept between 2017 and 2020. The statistical analysis used lactation milk yield, 305-day milk yield, lactation period, and daily milk yield as parameters to evaluate the association between genotype and milk yield traits. The data were analyzed using the general linear model in SPSS 25.0 software package (IBM Corp. 2017. SPSS 25.0 released 2017.). Environmental factors such as genotype, lactation order, and calving seasons were considered to have an impact on the relevant yield trait and were taken into account in the analysis.

According to the yield traits in the research, the following statistical model was employed.

$$Y_{ijkl}: \mu + a_i + b_j + c_k + e_{ijkl} \quad (1)$$

Where

Y_{ijkl} is any of the milk yield traits (lactation milk yield, 305-day milk yield, daily milk yield, and lactation period),

μ is the population mean,

a_i is the i^{th} genotype effect,

b_j is the effect of the j^{th} lactation order (j: 3; 1st Lactation: 1, 2nd Lactation: 2, 3rd Lactation: 3),

c_k is the effect of the k^{th} calving season (k: 2; 1: winter-spring, 2: summer-autumn),

e_{ijkl} is the error.

3. Results

The figures show the PCR amplification results and base sizes of PCR products Figure 1.



Figure 1. PCR results of the POU1F1 gene regions (260bp, with Standard GENESTA 100 bp DNA marker images, respectively).

A representative agarose gel image of the PCR-RFLP results for these polymorphisms under UV light is shown in Figure 2.

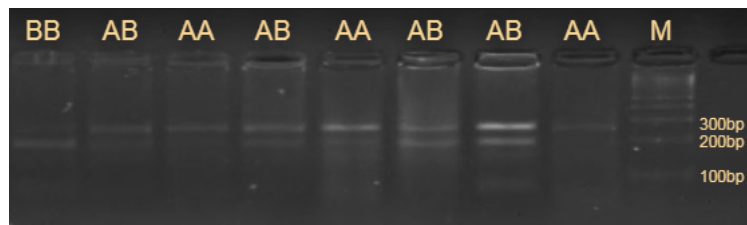


Figure 2. POU1F1/ *HinfI* restriction fragment size: AA; 260 bp, BB; 190 bp and 70 bp, AB; 260 bp, 190 bp, and 70 bp With Standard GENESTA 100 bp DNA marker.

Table 4. The genotype, allele frequencies, and H-W equilibrium for POU1F1/ *HinfI* genes

Genotype	N	allele A	allele B	Observed Frequency	Genotype Frequencies	H-W
AA	11	22	0	11	11.701	
AB	34	34	34	34	32.597	
BB	22	0	44	22	22.701	0.72 ^{ns}
Total	67	56	78	67		
p-value	134	0.42	0.58	134		

P-value for the test for Hardy-Weinberg equilibrium, in which $P > 0.05$ indicates that the sampled population is in Hardy-Weinberg equilibrium, ns: nonsignificant. (1 degree of freedom), H-W: Hardy Weinberg.

Allele and genotype frequencies of the remaining 67 Simmental DNA samples were calculated after excluding three samples in which the cut region could not be detected as a result of RFLP analyses. As a result of determining allele and genotype frequencies in Simmental cattle, it was found that the AA, AB, and BB genotype frequencies for the POU1F1 gene were 0.164, 0.508, and 0.328, respectively. The Hardy-Weinberg genetic equilibrium test showed that the distributions of POU1F1 genotype frequencies were in balance ($P > 0.05$) in the studied breed (Table 4).

Table 5 presents the values of milk performance traits for daily milk yields, corrected milk yields, and lactation periods in 100 Simmental herds in different lactations depending on the POU1F1/*HinfI* genotype.

As a result of the milk yield analyses we made in the Simmental cattle breed, whose numbers vary at different rates and have more than one lactation data on average, cows produced 4985 kg of milk, 5323 kg of real milk, and 17460 kg of milk over a 305-day period, with a lactation period of 285444 days. The AB genotype in the polymorphic regions resulted in the highest milk yield, while the AA genotype in the POU1F1/*HinfI* polymorphic region resulted in the lowest milk yield. However, the difference in milk yield between the highest and lowest genotypes was not significant. The second season range had the highest overall averages for all yield values, while the first season range had the

lowest averages. When considering lactation order, individuals in their 9th lactation had the highest average 305-day milk yield of 5658 kg, while those in their 4th lactation had the lowest average of 4898 kg. The highest daily milk yield average of 18,537 kg was found in individuals in their 9th lactation, while the lowest average of 16,099 kg was found in those in their 4th lactation. The highest real milk yield of 5270 kg was measured in the highest 6 lactations, while the lowest average of 4764 kg was found in the lowest 4 lactations. Lactation periods were longest in the highest 4 lactations at 302,229 days, and shortest in the 7th lactation period at 253,206 days (Table 5). In this study, there was no statistically significant difference between the milk yield averages of 100 Simmental cattle in 5 lactations and the average of genotype, calving season, and lactation order factors ($P > 0.05$).

Table 5. Effects of POU1F1/*HinfI* genotypes on milk yields in Simmental cattle

Variation Sources	N	Lactation milk yield	305-day milk yield	Daily milk yield	Lactation period	
		$\bar{X} \pm S\bar{X}$	$\bar{X} \pm S\bar{X}$	$\bar{X} \pm S\bar{X}$	$\bar{X} \pm S\bar{X}$	
POU1F1/ <i>HinfI</i>	AA	11	4569.145±514.017	5027.714±403.634	16.479±1.324	284.510±25.558
	AB	63	5546.732±246.071	5651.558±193.229	18.545±0.634	304.344±12.235
	BB	26	4841.260±339.780	5291.175±266.814	17.355±0.875	267.477±16.894
	P		0.070	0.234	0.228	0.148
Calving season	1	62	4771.295±296.357	5128.166±232.716	16.806±0.764	281.655±14.735
	2	38	5200.130±313.769	5518.798±246.389	18.114±0.808	289.23±15.601
	P		0.254	0.187	0.178	0.685
	3	3	4823.382±938.570	5222.156±737.017	17.129±2.418	285.844±46.667
Lactation order	4	8	4764.374±580.059	4898.207±455.494	16.099±1.494	302.229±28.841
	5	17	5198.082±401.742	5447.053±315.470	17.87±1.035	294.281±19.975
	6	22	5270.972±361.534	5507.864±283.897	18.055±0.931	298.698±17.976
	7	25	4811.469±349.726	5374.027±274.624	17.620±0.901	253.206±17.389
	8	17	4963.763±409.244	5156.588±321.361	16.899±1.054	296.910±20.348
	9	8	5067.945±613.693	5658.480±481.905	18.537±1.581	266.939±30.514
P		0.960	0.878	0.884	0.435	
Total	100	4985.713±241.234	5323.482±189.430	17.460±.62	285.444±11.995	

\bar{X} : General Means, $S\bar{X}$: Standard deviation.

As a result of the milk yield analyses we made in the Simmental cattle breed, whose numbers vary at different rates and have more than one lactation data on average, cows produced 4985 kg of milk, 5323 kg of real milk, and 17.460 kg of milk over a 305-day period, with a lactation period of 285.444 days. The AB genotype in the polymorphic regions resulted in the highest milk yield, while the AA genotype in the POU1F1/*HinfI* polymorphic region resulted in the lowest milk yield. However, the difference in milk yield between the highest and lowest genotypes was not significant. The second season range had the highest overall averages for all yield values, while the first season range had the lowest averages. When considering lactation order, individuals in their 9th lactation had the highest average 305-day milk yield of 5658 kg, while those in their 4th lactation had the lowest average of 4898 kg. The highest daily milk yield average of 18.537 kg was found in individuals in their 9th lactation, while the lowest average of 16.099 kg was found in those in their 4th lactation. The highest real milk yield of 5270 kg was measured in the highest 6 lactations, while the lowest average of 4764 kg was found in the lowest 4 lactations. Lactation periods were longest in the highest 4 lactations at 302.229 days, and shortest in the 7th lactation period at 253.206 days (Table 5). In this study, there was no statistically significant difference between the milk yield averages of 100 Simmental cattle in 5 lactations and the average of genotype, calving season, and lactation order factors ($P > 0.05$).

4. Discussion

The POU1F1/*HinfI* gene has been studied in several populations of Simmental cattle, with varying results in terms of allele frequencies. In Table 1, we can see the frequencies of the A and B alleles in different populations, as reported in previous studies. Our study found that the frequency of the dominant B allele for POU1F1/*HinfI* in Simmental cattle was 0.58, while the frequency of the

recessive A allele was 0.42. This suggests that the B allele is more common in our population than the A allele.

Comparing our results to those in Table 1, we can see that the frequency of the B allele in our population is similar to that reported in some studies, while it is higher than in others. The frequency of the A allele in our population is also similar to that reported in some studies, while it is lower than in others. Overall, our study provides new information on the allele frequencies of the POU1F1/HinfI gene in Simmental cattle, which can be used to better understand the genetics of this population.

We performed a Hardy-Weinberg equilibrium test to assess the balance of genotype frequencies in the herd. Our results indicated that the genotype frequencies for POU1F1/HinfI were in balance ($P > 0.05$), likely due to the closed barn environment, absence of external migration, and controlled breeding practices. Similarly in our study, it has been reported that POU1F1/HinfI genotype frequencies are in Hardy-Weinberg equilibrium in Holstein calves, Anatolian Black Steppe, and Turkish Grey cattle (Ozdemir et al., 2018; Cobanoglu and Ardicli, 2022; Findik and Özdemir, 2022; Sakar and Zülkadir, 2022; Sadeghi et al., 2022), and similarly, genotype distributions are balanced in Holstein Friesian, Brown Swiss, and Simmental cattle breeds (Mohammadabadi et al., 2015; Aytekin and Bayraktar, 2022). On the other hand, in different cattle breeds such as Grati-Ongole, Bos Indicus, and Bali cattle (*Bos javanicus*), it has been reported that Pit-1 genotype distributions are not in Hardy-Weinberg equilibrium and there are deviations in HW equilibrium (Jakaria and Noor, 2015; Hartati et al., 2018).

In our study, due to the limited size of our Simmental breed and milk yield records, we were unable to establish any significant correlation between POU1F1/HinfI genotype polymorphisms and milk yield ($P > 0.05$). Similar studies on different breeds, such as buffaloes, Slovak Simmental, and Holstein cattle, have also reported no significant association between these genotypes and milk yield (Heidari et al., 2012; Ebrahimi et al., 2015; Trakovická et al., 2015; Ozdemir et al., 2018; Thuy et al., 2018; Zghair and Hassooni, 2021). However, studies conducted in the Horasan region of Iran reported that the POU1F1/HinfI genotype polymorphism is linked to milk composition in Holstein cattle, affecting protein and fat percentages, and in Shawial cattle, affecting total milk yield and 300-day milk yield (Chauhan et al., 2015; Mohammadabadi et al., 2015; Sadeghi et al., 2022) Further research with larger sample sizes and more diverse breeds is necessary to better understand this relationship.

Studies investigating the association of POU1F1 gene polymorphisms have not only focused on milk yield but also on growth and reproductive traits in various cattle breeds. Specifically, research on Polish Black-and-White Holstein-Friesian and Limousine cattle and calves has found that Pit-1 gene polymorphisms are associated with body weight, age at sexual maturity, and reproductive parameters such as insemination index (Pytlewski et al., 2018; Pytlewski et al., 2022). However, studies on Pit-1/HinfI polymorphisms and growth parameters have yielded mixed results, with some studies reporting no association with birth weight in calves, growth in Black Anatolian cattle, and growth and reproductive parameters in Canchim cattle (Grossi et al., 2015; Findik and Özdemir, 2022; Sakar and Zülkadir, 2022).

Conclusion

In association analysis studies published to determine the potential of the gene polymorphisms to be used as markers in cattle breeding, the above-mentioned genes appear to have significant implications for body weight, meat yield, and reproductive performance in general. There is still much to learn about the effects of POU1F1 gene polymorphisms on Simmental cattle performance traits. Future research should focus on expanding the sample size to increase the statistical power of the study. Additionally, studies should examine other genetic factors that may interact with these genes to influence performance traits. Finally, more research is needed to determine how these findings can be applied in practical breeding programs to improve Simmental cattle performance as markers in milk data analyses.

Ethical Protocol

Ethical Protocol was approved by the experimental animal ethics committee of Atatürk University with its session dated 27.10.2022 and decision numbered 257.

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