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Research Article

Meta Analysis of Allele and Genotype Frequency of Growth Hormone (*b*GH) Gene *Alu*I Polymorphism, Which is Effective on Milk Yield in Holstein Cattle [1]

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Abstract

There are studies investigating the relationship between *b*GH gene *Alu*I polymorphism and milk yield in bovine breeds. In the presented study, the relationship between different genotypes and lactation milk yields was determined by analyzing *b*GH-*Alu*I genotype and allele frequencies in Holstein breed by meta-analysis method. The study material consisted of studies investigating bGH-AluI polymorphisms that are effective on milk yield in Holstein breed. According to the determined criteria, allele and genotype frequencies of 4583 samples in 20 studies selected from 35 studies and milk yields of 932 samples in 6 studies in which milk yields were calculated according to genotypes were included in the meta analysis. In the study, meta analyzes LL, LV and VV genotypes and L and V alleles and lactation milk yields were performed for bGH gene. The study samples were proved to be unbiased with the Begg and Mazumdar Rank Correlations test and funnel plot. As a result of the study, high heterogeneity was determined between the investigated studies concerning all genotypes (LL, LV and VV) and allele frequencies (L and V), thus random effect model was used. Meta analyzes were done with Comprehensive Meta-Analysis Software (CMA). According to the random effect model, pooled ratios in LL, LV and VV genotypes are 0.710, 0.260 and 0.030; pooled ratios for L and V allele frequencies were calculated as 0.818 and 0.182 and were found statistically significant (P<0.001). In addition, mean milk yields in LL, LV and VV genotypes were calculated as 6.64, 6.96, 7.13 L x1000 according to meta-analysis and subgroup analysis results, but no significant difference was found (P=0.958). At the end of the study, closer results to the population parameter were obtained regarding the relationship between bGH-AluI polymorphism genotype and milk yield in Holstein cattle with meta-analysis.

Keywords: *AluI, bGH gene, Holstein, Meta analysis, Milk yield*

Holştayn Irkı Sığırlarda Süt Verimi Üzerine Etkili Büyüme Hormonu (*b*GH) Geni *Alu*I Polimorfizminin Allel ve Genotip Frekanslarının Meta Analizi

Öz

Sığır ırklarında *b*GH geni *Alu*I polimorfizmi süt verim ilişkilerinin araştırıldığı çalışmalar bulunmaktadır. Sunulan çalışmada Holstayn ırkında *b*GH-*Alu*I genotip ve allel frekanslarının meta analizi yöntemi ile analiz edilerek, farklı genotipler ve laktasyon süt verimleri arasındaki ilişki belirlenmiştir. Çalışma materyalini, Holştayn ırkında süt verimi üzerine etkili *bGH-Alu*I polimorfizmlerinin araştırıldığı çalışmalar oluşturmuştur. Belirlenen kriterlere göre 35 çalışma içerisinden seçilen 20 çalışmadaki toplam 4583 örneğin allel ve genotip frekansı ve seçilen çalışmalar içerisinde genotiplere göre süt verimlerin hesaplandığı 6 çalışmadaki 932 örneğin süt verimleri meta analizine dahil edilmiştir. Çalışmada bGH geni LL, LV ve VV genotipleri, L ve V allellerinin frekansları ve laktasyon süt verimleri için meta analizleri yapılmıştır. Çalışma örneklemlerinin yanlı olmadığı Begg ve Mazumdar Sıra Korelasyonları testi ve huni grafiği ile kanıtlanmıştır. Çalışma sonucunda, tüm genotiplerde (LL, LV ve VV) ve allel frekanslarında (L ve V) incelenen çalışmalar arasında yüksek heterojenite belirlenmiş, bu nedenle rassal etki modeli kullanılmıştır. Meta analizleri Comprehensive Meta-Analysis Software (CMA) ile yapılmıştır. Rassal etki modeline göre LL, LV ve VV genotiplerinde ortak frekanslar 0.710, 0.260 ve 0.030; L ve V allel frekansları için ortak frekanslar 0.818 ve 0.182 olarak hesaplanmış ve istatistiksel olarak önemli bulunmuştur (P<0.001). Ayrıca, LL, LV ve VV genotiplerinde ortalama süt verimleri meta analizi ve alt grup analizi sonuçlarına göre 6.64, 6.96, 7.13 L x1000 olarak hesaplanmış ancak anlamlı farklılık bulunmamıştır (P=0.958). Çalışma sonucunda yapılan meta analizi ile Holştayn ırkı sığırlarda bGH-AluI polimorfizminin genotip ve süt verimi arasındaki ilişkileri hakkında popülasyon parametresine daha yakın sonuçlar elde edilebilmiştir.

Anahtar sözcükler: *AluI, bGH geni, Holştayn, Meta analizi, Süt verimi*

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INTRODUCTION

Growth hormone secreted from the anterior pituitary gland and functioning in many different physiological processes such as milk yield, growth, fertility in farm animals is a protein-nature hormone with a single chain polypeptide structure consisting of 191 amino acids [1-3]. In cattle, the growth hormone (bGH) gene, localised in chromosome 19, is 1793 base pairs and consists of 5 exons and 4 introns [4]. Because it plays an important role in physiological processes, the bGH gene has been reported to be a potential candidate gene for growth and live weight gain, milk yield, fertility and embryo quality in farm animals [2]. Studies conducted by different researchers especially in terms of milk yield characteristics have revealed a relationship between different SNP regions in the bGH gene and milk yield characteristics [4-6].

In recent years, rapid developments in molecular technologies and molecular genetic applications have been used in the breeding and selection of livestock. When cost-benefit comparison is made between molecular genetic analysis methods, restriction fragment length polymorphism (RFLP) method has significant advantages. Bacterial restriction enzymes, (restriction endonuclease) are the enzymes that make double-strand breaks in DNA at specific sites by recognizing specific nucleotides of target genes on double-stranded DNA [7].

Restriction enzymes play a role in the defense mechanism of bacteria by breaking DNA outside the bacterial genome from specific nucleotide regions [8]. In RFLP studies, these specific enzymes produced by bacteria are isolated and used. Restriction enzymes used in RFLP studies are named using the names of the bacteria from which they were isolated [9]. The *Alu*I restriction enzyme used in the bGH-*Alu*I polymorphism studies selected in this study was obtained from *Arthrobacter luteus* [9].

There are many studies investigating bGH-*Alu*I polymorphism and the relationship between this polymorphism and milk yield characteristics in cattle [4,5,10]. Different results are found in many studies investigating the relationship between bGH-*Alu*I polymorphism and milk yield in different Holstein populations. Although different findings obtained from several studies on the same subject have been reviewed in reviews, meta-analysis method has been developed to combine the results and make them a common finding by using statistical methods. Meta-analysis is carried out to increase the precision and power of the parameter estimation by combining the results of the studies conducted with small samples to increase the sample width in a consistent and coherent manner [11].

Today, meta-analysis has found widespread use in many fields such as medicine, veterinary, biology, agriculture, psychology, education and its importance has increased gradually. In recent years, meta-analysis studies have also been found in the field of genetics [12-14].

In this study, it was aimed to determine the heterogeneity between the studies and calculate pooled ratios by analyzing the results concerning the genotype and allele frequencies obtained from the studies investigating bGH-*Alu*I polymorphism in Holstein breed cattle with the meta-analysis method.

According to the pooled genotype and allele frequency ratios calculated from the studies included in the meta-analysis, the bGH-*Alu*I polymorphism Hardy-Weinberg χ^2 test results for the entire population were calculated.

Additionally, meta-analysis and subgroup analyzes of the mean yield values of studies determining lactation milk yields according to genotypes were performed and milk yields were compared according to genotypes.

MATERIAL and METHODS

The literature to be included in the meta analysis in our study have been accessed by searching the PubMed, Web of Science and Google Scholar databases using the keywords "bGH gene", "*Alu*I restriction enzyme" and "Holştayn-Holstein". The study material consisted of 20 studies selected from 44 primitive studies conducted between 2002 and 2018 (See supplementary table for references). The criteria for inclusion in the meta analysis were use of Holstein cattle as animal material as well as *Alu*I restriction enzyme in genetic analysis, and determining genotype (LL, LV and VV) and allele (L and V) frequencies in terms of bGH-*Alu*I polymorphism. In addition, subgroup analyzes were performed according to the genotypes used for meta-analysis of the yield averages of 6 studies investigating the relationship between genotype and lactation milk yields in terms of bGH-*Alu*I polymorphism. After determining the studies that met the criteria for inclusion in the meta-analysis, Begg and Mazumdar Rank Correlation test was used to determine whether the study sample is unbiased. The heterogeneity of the effect sizes between studies was evaluated in order to decide the meta-analysis method (fixed/random effect model) to be used to combine the results of the study and calculate the pooled ratios. Cochran's Q test statistics, I^2 statistics and τ^2 statistics were calculated to determine the heterogeneity between studies, to determine the level of heterogeneity and to determine the true variance between studies respectively. The simplest and most common approach used to assess whether there is true heterogeneity between studies is the (k-1) degree of freedom Chi-Square heterogeneity test proposed by Cochran, which is known as the Q statistic and is calculated as follows:

$$Q = \sum_{i=1}^{k} w_i Y_i^2 - \frac{\left(\sum_{i=1}^{k} w_i Y_i\right)^2}{\sum_{i=1}^{k} w_i}$$

It is also referred to as the standard χ2 test in the literature. I^2 statistic can be used to find out to what extent the observed variance reflects the true variance in effect size. I^2 is calculated as follows:

$$I^2 = \left(\frac{Q - df}{Q}\right) \times \%100$$

τ^2 the Tau-square coefficient (τ^2) is the variance of the true effect size and is calculated as follows:

$$\hat{\tau}^2 = \begin{pmatrix} \dfrac{Q - (k-1)}{\sum w_i - \dfrac{\sum w_i^2}{\sum w_i}}, & Q > (k-1) \\ 0, & Q \leq (k-1) \end{pmatrix}$$

Effect size indicates the magnitude of the result of an exposure [12]. The random effect model (Der Simonian-Laird method) was used in the analyses because the effect sizes were heterogeneous among the studies. The random effect model takes into account the variance of studies both within and between studies and assumes differences in effect size among all studies. All of the meta analyzes in the study were done with Comprehensive Meta-Analysis Software (CMA).

The genotype and allele frequencies of the bGH-AluI polymorphism of the studies included in the meta analysis are given in *Table 1*.

Means of milk yield (x1000, Liter) in studies in which lactation milk yield are determined according to bGH-*Alu*I polymorphism genotypes are given in *Table 2*.

RESULTS

In the study, genotype and allele frequencies in terms of bGH-AluI polymorphism belonging to 4583 head Holstein cattle in 20 studies conducted between 2002-2018 were included in the meta-analysis. The ratios of the frequencies of the genotypes and alleles determined in these studies and the Hardy-Weinberg (HW) χ^2 test results of the populations are given in *Table 3*. Thus, it was determined that the populations of 9 studies in terms of investigated genotype were not in HW equilibrium (P<0.05).

A total of 4583 Holstein cattle examined in 20 studies were included in the meta-analysis of LL, LV and VV genotypes and L and V alleles in the present study. Cochran's Q, I^2 and τ^2 test statistics were calculated to evaluate heterogeneity between studies for all genotype and allele frequency ratios. According to the calculated I^2 (%) values, high hetero-geneity was detected in all genotype and allele frequency ratios.

Table 1. *Genotype and allele frequencies of studies included in meta analysis*

No	Study	Year	Country	n	Genotype Frequency			Allel Frequency	
					n(LL)	n(LV)	n(VV)	n(L)	n(V)
1	Dybus et al.	2002	Poland	1086	709	352	25	885	201
2	Biswas et al.	2002	India	33	24	8	1	28	5
3	Sørensen et al.	2002	Denmark	415	311	85	19	354	61
4	Kováks et al.	2006	Hungary	363	314	47	2	338	25
5	Zakizadeh et al.	2006	Iran	110	79	29	2	17	94
6	Pawar et al.	2007	India	55	30	22	3	41	14
7	Balogh et al.	2008	Hungary	22	18	4	0	20	2
8	Hradecká et al.	2008	Germany	315	288	27	0	301	14
9	Balogh et al.	2009	Hungary	307	247	57	3	275	32
10	Mohammadabadi et al.	2010	Iran	103	56	32	15	72	31
11	Misrianti et al.	2012	Indonesia	370	329	41	0	348	22
12	Heidari et al.	2012	Iran	100	78	21	1	88	12
13	Akyuz et al.	2013	Turkey	150	116	9	26	126	24
14	Hadi et al.	2015	Iran	150	59	92	0	104	47
15	Hartatik et al.[a]	2015	Indonesia	19	16	3	0	17	2
16	Hartatik et al.[b]	2015	Indonesia	43	34	9	0	39	4
17	Molee et al.	2015	Thailand	231	0	198	33	132	99
18	Ozdemir et al.	2017	Turkey	186	93	89	4	138	48
19	Sonmez et al.	2018	Turkey	115	47	56	12	75	40
20	Amiri et al.	2018	Tunisia	410	319	76	15	357	53
Total				**4583**	**3166**	**1256**	**161**	**3754**	**829**

Table 2. *Mean of milk yields (x1000, Liter) of bGH-AluI polymorphism genotypes*

No	Study	Year	LL		LV		VV	
			n	X±Sx	n	X±Sx	n	X±Sx
1	Kováks et al.	2006	314	10.01±1.48	47	10.33±1.59	2	10.16±1.31
2	Pawar et al.	2007	30	3.16±0.09	22	3.89±0.08	3	3.80±0.05
3	Hadi et al.	2015	59	9.32±0.54	92	8.98±0.52	-	-
4	Hartatik et al.[a]	2015	16	4.00±1.56	3	5.19±0.23	-	-
	Hartatik et al.[b]	2015	34	5.66±1.83	9	5.79±1.49	-	-
5	Ozdemir et al.	2017	93	7.02±0.39	89	7.16±0.37	4	7.01±0.66
6	Sönmez et al.	2018	47	7.27±0.16	56	7.35±0.15	12	7.84±0.27

Table 3. *The studies included in the meta analysis and genotype and allele ratios of the herds examined*

Study	Genotype			Allel		HW χ^2 Values	P Values
	LL	LV	VV	L	V		
Dybus et al.	0.653	0.324	0.023	0.815	0.185	6.029	**P<0.05**
Biswas et al.	0.730	0.240	0.030	0.850	0.150	0.108	P>0.05
Sørensen et al.	0.749	0.205	0.046	0.852	0.148	14.780	**P<0.05**
Kováks et al.	0.865	0.130	0.005	0.930	0.070	0.028	P>0.05
Zakizadeh et al.	0.720	0.260	0.020	0.150	0.850	0.126	P>0.05
Pawar et al.	0.540	0.400	0.060	0.746	0.254	0.160	P>0.05
Balogh et al.	0.826	0.166	0.008	0.909	0.091	0.220	P>0.05
Hradecká et al.	0.914	0.086	0	0.957	0.043	0.632	**P<0.05**
Balogh et al.	0.803	0.186	0.011	0.896	0.104	0.021	P>0.05
Mohammadabadi et al.	0.544	0.311	0.146	0.699	0.301	7.051	**P<0.05**
Misrianti et al.	0.890	0.110	0	0.940	0.060	1.273	P>0.05
Heidari et al.	0.781	0.205	0.013	0.884	0.116	0.100	P>0.05
Akyuz et al.	0.770	0.060	0.170	0.840	0.160	100.326	**P<0.05**
Hadi et al.	0.390	0.610	0	0.690	0.310	28.981	**P<0.05**
Hartatik et al.[a]	0.840	0.160	0	0.920	0.080	0.140	P>0.05
Hartatik et al.[b]	0.790	0.210	0	0.900	0.100	0.587	P>0.05
Molee et al.	0	0.857	0.143	0.572	0.428	129.938	**P<0.05**
Ozdemir et al.	0.500	0.480	0.020	0.740	0.260	10.818	**P<0.05**
Sonmez et al.	0.410	0.490	0.100	0.652	0.348	0.618	P>0.05
Amiri et al.	0.778	0.185	0.037	0.870	0.130	12.783	**P<0.05**

Forest plots related to meta-analysis of genotypes and alleles is shown in *Fig. 1*. As a result of meta analysis, LL genotype pooled ratio of 0.710 (0.633-0.777) was calculated higher than LV and VV genotypes and L allele pooled ratio of 0.818 (0.751-0.871) was calculated higher than V allele *(Table 4)*.

Kendall's Tau-b correlation coefficient was calculated in the Begg and Mazumdar rank correlations statistics to determine publication bias. This coefficient is expected to be close to 1 and the p value is expected to be greater than 0.05. The results of Begg and Mazumdar Rank Correlations test revealed that there was no publication bias in the study samples for all genotypes and allele frequency ratios (P>0.05) *(Table 5)*. In the *Fig. 2*, funnel plots related to study sample of LL, LV and VV genotypes and L, V alleles are shown.

According to the genotype pooled ratios calculated as a result of meta analysis, the frequencies determined for all populations (n=4583) weren't found in the Hardy-Weinberg equilibrium in terms of bGH-AluI polymorphism. (χ^2=6.804, P=0.009) *(Table 6)*.

In the study, secondly, meta-analysis was performed with 6 studies in which the means of lactation milk yield were determined in the genotypes of bGH-AluI polymorphism, and the mean milk yields of the genotype subgroups were compared. High heterogeneity was determined between studies according to mean of milk yields (Q=60926.647, P<0.001, I^2=99.972). Therefore, random effect model was chosen in meta analysis and the overall mean of milk yield in the studies was found as 6.86 (5.83-7.89) L x1000. As a

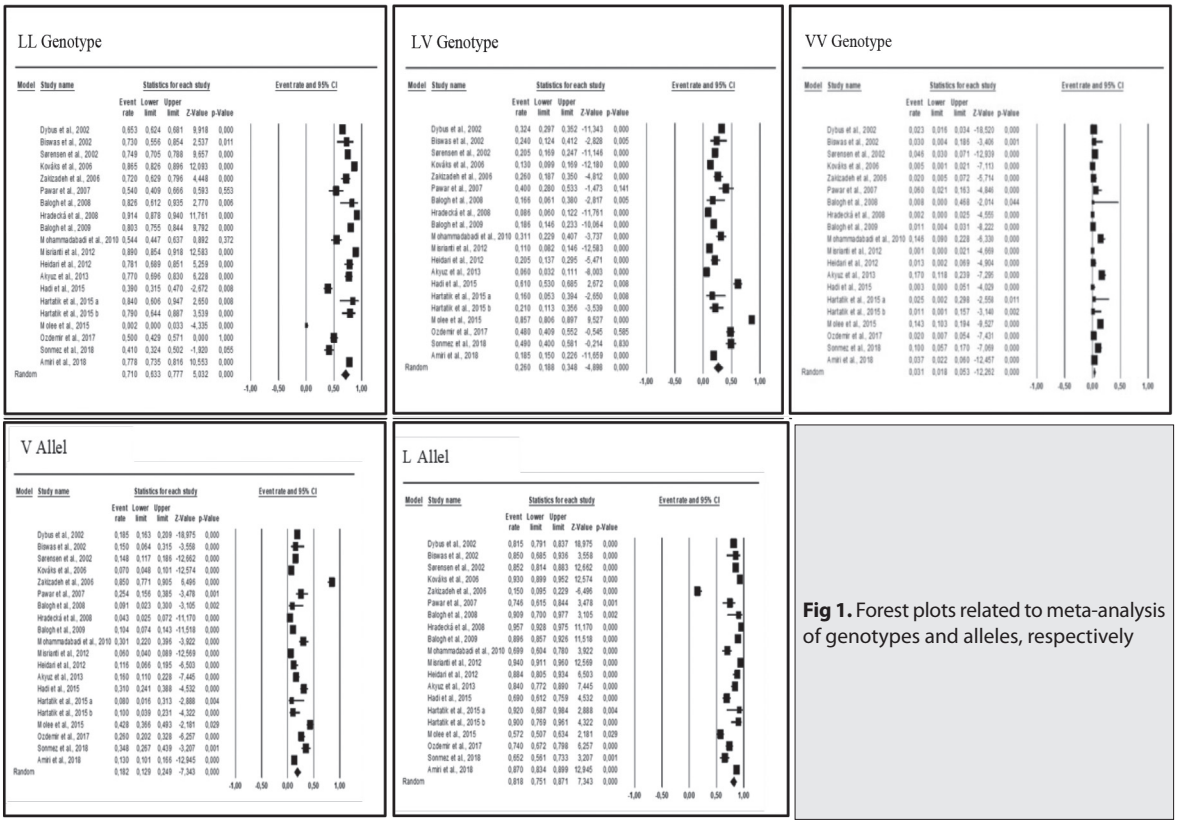

Fig 1. Forest plots related to meta-analysis of genotypes and alleles, respectively

Table 4. *Assessment of heterogeneity between studies for genotype and allele frequencies and calculated pooled ratios*

Variables		Genotype and Allele Frequency Ratios			Heterogeneity			
		Pooled Ratio	95% Confidence Interval	P Values	Cochran's Q	P Values	I^2 (%)	τ^2
Genotype	LL	0.710	0.633-0.777	<0.001	386.335	<0.001	95.082	0.545
	LV	0.260	0.188-0.348	<0.001	565.344	<0.001	96.639	0.837
	VV	0.030	0.018-0.053	<0.001	154.038	<0.001	87.665	1.034
Allel	L	0.818	0.751-0.871	<0.001	402.647	<0.001	95.281	0.743
	V	0.182	0.129-0.249	<0.001	402.647	<0.001	95.281	0.743

n: *Total number of cattle inclded in meta analysis,* ***Q:*** *Weighted sum of squares of observed effect sizes* I^2***:*** *The proportion of true variance to observed variance,* τ^2***:*** *Estimate of the between-study variance in true effects*

Table 5. *Publication bias tests of genotype and allele frequencies studies*

Variables		Begg and Mazumdar Rank Correlation Test	
		Kendall's Tau-b Correlation Coefficient	P Values
Genotype	LL	-0.068	0.673
	LV	-0.026	0.871
	VV	-0.100	0.538
Allel	L	0.079	0.627
	V	-0.079	0.627

result of the Begg and Mazumdar rank correlation test, it was observed that there was no publication bias in the study sample regarding mean of milk yields (P=0.325). The mean of milk yields for LL, LV and VV genotypes were calculated as 6.64, 6.96, 7.13 (x1000, Liter), respectively with the subgroup analysis. However, in subgroup analysis, there was no significant difference between genotypes for mean of milk yields (Q= 0.086, df= 2, P=0.958) *(Table 7)*. Forest plot of subgroup analysis of milk yield's (x1000, Liter) mean according to genotypes is shown in *Fig. 3*.

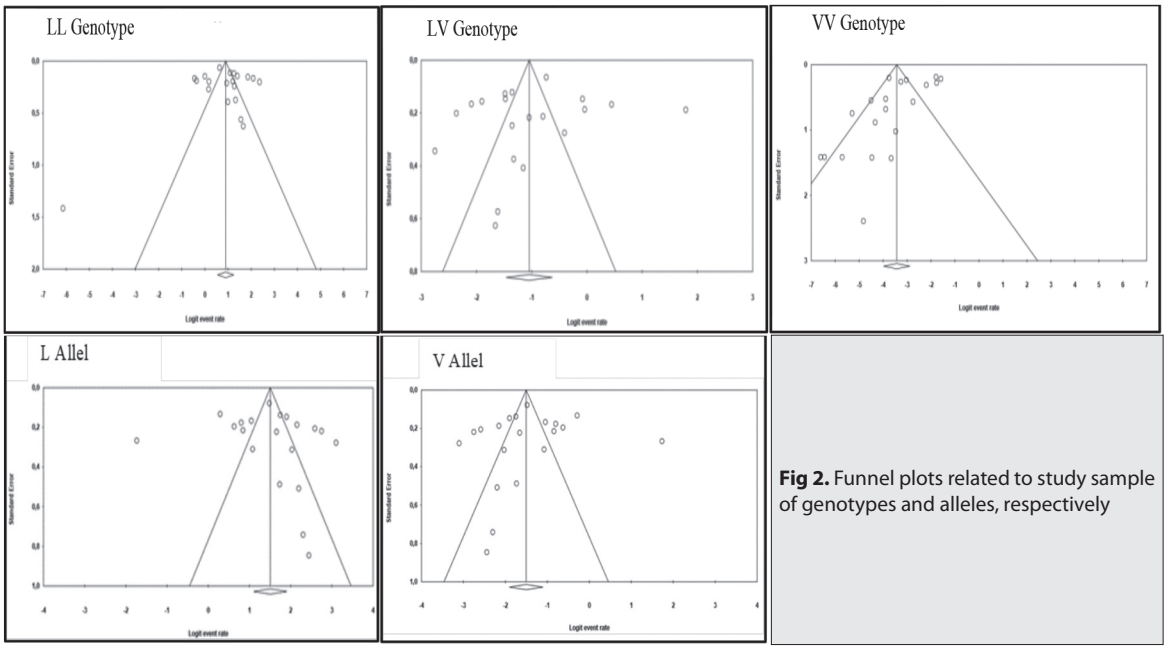

Fig 2. Funnel plots related to study sample of genotypes and alleles, respectively

Table 6. *Test of consistency with Hardy-Weinberg equilibrium in terms of bGH-AluI polymorphism according to the calculated genotype pooled ratios*

Statistics	Genotype			Statistical Significant
	LL	LV	VV	
Pooled Ratios (%)	0.710	0.260	0.030	χ^2=6.804 P=0.009 (df=1)
Calculated Frequencies (n)	3166	1256	161	

Table 7. *Subgroup analysis results and mean of milk yields (x1000, Liter) by genotypes*

Genotype	Number of Study	Mean of Milk Yields	95% Confidence Interval of Milk Yields
LL	7	6.64	4.50-8.78
LV	7	6.96	5.21-8.71
VV	4	7.13	4.32-9.94

Model	Group by Genotype	Study name	Statistics for each study						Mean and 95% CI
			Mean	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
	LL	Kováks et al., 2006 a	10003,010	83,323	6942,653	9839,701	10166,319	120,052	0,000
	LL	Pawar et al., 2007 a	3156,000	16,614	276,033	3123,437	3188,563	189,957	0,000
	LL	Hadi et al., 2015 a	9317,000	70,562	4979,051	9178,700	9455,300	132,039	0,000
	LL	Hartatik et al., 2015 a 1	4000,000	390,000	152100,000	3235,614	4764,386	10,256	0,000
	LL	Hartatik et al., 2015 b 1	5660,000	313,842	98497,059	5044,880	6275,120	18,035	0,000
	LL	Ozdemir et al., 2017 a	7023,800	40,804	1664,970	6943,826	7103,774	172,135	0,000
	LL	Sonmez et al., 2018 a	7274,500	22,770	518,451	7229,873	7319,127	319,484	0,000
Random	LL		6641,275	1091,484	1191338,299	4502,005	8780,545	6,085	0,000
	LV	Kováks et al., 2006 b	10334,660	232,143	53890,222	9879,669	10789,651	44,519	0,000
	LV	Pawar et al., 2007 b	3895,000	16,203	262,545	3863,242	3926,758	240,384	0,000
	LV	Hadi et al., 2015 b	8979,000	54,005	2916,565	8873,152	9084,848	166,262	0,000
	LV	Hartatik et al., 2015 a 2	5190,000	132,791	17633,333	4929,735	5450,265	39,084	0,000
	LV	Hartatik et al., 2015 b 2	5790,000	496,667	246677,778	4816,551	6763,449	11,658	0,000
	LV	Ozdemir et al., 2017 b	7157,200	39,294	1544,028	7080,185	7234,215	182,144	0,000
	LV	Sonmez et al., 2018 b	7354,600	20,272	410,944	7314,868	7394,332	362,800	0,000
Random	LV		6960,369	890,472	792940,648	5215,076	8705,663	7,816	0,000
	VV	Kováks et al., 2006 c	10158,000	925,263	856112,295	8344,517	11971,483	10,978	0,000
	VV	Pawar et al., 2007 c	3803,000	31,177	972,000	3741,894	3864,106	121,981	0,000
	VV	Ozdemir et al., 2017 c	7007,700	331,100	109627,210	6358,756	7656,644	21,165	0,000
	VV	Sonmez et al., 2018 c	7836,900	78,635	6183,480	7682,778	7991,022	99,662	0,000
Random	VV		7128,383	1432,456	2051929,721	4320,822	9935,945	4,976	0,000
Random	Overall		6888,509	621,626	386418,667	5670,145	8106,873	11,081	0,000

Fig 3. Forest plot of subgroup analysis of milk yields's (x1000, Liter) mean by genotypes

Suplementary Table. *References of studies included in meta analysis*	
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DISCUSSION

The genetic structure of the population is defined by the frequency of the genotypes present in the population and the frequency of the alleles of a gene, in terms of the feature under consideration [15]. It is necessary to study large samples in determining the genetic structure because when working with small samples, the probability of statistically significant differences between genotypes decreases. However, in cases where it is necessary to work with a small sample, the same hypothesis needs to be repeated at different times or by different researchers, and if the number of research exceeds 4, the results can be re-evaluated by meta-analysis. The results obtained by meta analysis can be used in the sample size calculation of new researches and comparisons can be made [16,17].

Since it has the potential to be a marker and is the most common polymorphism in the Holstein breed, in this study, genotype and allele frequencies and relations with milk yield characteristics obtained from studies investigating the bGH-*Alu*I polymorphism revealed by *Alu*I restriction enzyme in Holstein cattle were meta-analized. In terms of bGH-AluI polymorphism performed in Holstein cattle breeds, 4583 cattle in 20 studies on LL, LV and VV genotypes and L and V allell frequency ratios were included in the meta-

analysis. Since the high heterogeneity was determined between studies in all genotypes and allele frequencies, the DerSimonian-Laird method was used in calculating pooled ratios, because the random effect model was least affected by the outlier value.

According to the calculated pooled ratios, the LL genotype frequency ratio is highest (0.710); The VV genotype frequency ratio was the lowest (0.030). Thus, the L allele frequency pooled ratios (0.818) was calculated higher than the V allele frequency ratio (0.182). These results were similar to those of the studies included in the meta-analysis. The LL genotype frequency ratio was highest in most of the studies (17/20); included in the meta-analysis except three studies [18-20]. The VV genotype frequency ratio was reported to be higher than the LV genotype frequency ratio in one study [21] and the lowest in others (18/20). In addition, VV genotype could not be detected in five studies. It has been reported that the frequency ratio of the L allele is higher than the frequency ratio of the V allele in nineteen studies.

Hardy-Weinberg equilibrium is the basis of genetic inferences in the field of population genetics. According to the Hardy-Weinberg equilibrium, genotype frequencies do not change from generation to generation in populations that do not have selection, mutation, migration, and randomly mating. The Hardy-Weinberg equilibrium for samples randomly taken from a population is determined by the Pearson's $\chi 2$ compatibility test. In cases where the sample size is not sufficient, the prerequisites for suitability tests cannot be fulfilled and the reliability of the obtained results is low [22,23]. In this study, it was observed that 5 of the 20 studies included in the meta-analysis had been performed with a small sample size (<55). The bGH-AluI polymorphism genotype frequencies of the majority of studies (11/20) were found to be compatible with the Hardy-Weinberg equilibrium [23]. As the result of meta analysis, it was seen that all studies combined (n=4583) were in Hardy-Weinberg equilibrium those genotype frequencies were determined according to the calculated pooled ratios (P=0.009). The amount of heterozygosity determined in a population is the most important feature used in determining the genetic diversity of that population [15]. The variation in heterozygosity (LV) values observed in studies included in the meta-analysis (0.060-0.857) was found to be quite wide. This ratio was calculated as 0.260 as a result of meta analysis. Thus, closer results to the population parameter could be obtained with a large sample.

In previous studies, it has been reported that bGH-*Alu*I polymorphism in bGH gene, which is accepted as a potential candidate gene for genetic selection studies in Holstein cattle, has effects on meat and milk yield characteristics. In addition, studies have also been conducted in Holstein cattle to report that this polymorphism is associated with increased body weight [24,25]. Different results were obtained in the studies investigating the effect of bGH gene genotypes on milk yield characteristics in Holstein cattle. Many studies have reported that milk yield characteristics do not vary with respect to genotypes [18-20,26-28]. However, in some studies, cattle with LL genotypes were reported to have higher milk yield [4,29], whereas in two studies reported higher milk yield in cattle with LV genotype [5,30]. In the present study, it was determined by meta-analysis that the relationship between bGH-*Alu*I polymorphism genotypes and milk yield in different Holstein populations did not differ statistically. Meta-analysis is a statistical method used to systematically compile the results of scientific research carried out independently of each other and to combine these results with quantitative methods and present them as a summary of finding. This method has increased significantly and its use has become widespread day by day.

In this study, the relationship between genotype and allele frequencies and milk yield from the studies investigating bGH-*Alu*I polymorphism in Holstein cattle was synthesized by meta-analysis. Thus, closer results to the population parameter could be obtained in the phenotype-genotype relationships. It is thought that the results obtained in the present study will contribute to the development of new strategies required for rational production in dairy cattle.

However, within the scope of the study, more articles should be obtained in order to make the results more reliable as well as in order to compare a large number of groups on the result variability and to rank among the groups, the application of newly developed network meta-analysis methods is of importance for breeding. It is necessary to carefully select and analyze the studies to be included in the analysis in order to obtain correct results in applications and also to use the appropriate statistical model and to interpret the obtained analysis results correctly.

Conflict of Interest

The authors declare that there are no conflicts of interest

Statement of Author Contributions

Conception and design: A. Akçay, B. Akyüz Acquisition of data: A. Akçay, F. Daldaban, E. Çelik. Analysis and interpretation of data: A. Akçay, E. Çelik. Drafting the article: A. Akçay, K. Arslan, B. Akyüz. Revising it for intellectual content: A. Akçay, K. Arslan, B. Akyüz. Final approval of the completed article: A. Akçay, F. Daldaban, E. Çelik, K. Arslan, B. Akyüz

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