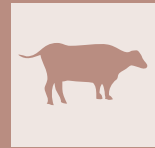# The relationships between *PRL/Rsa I* polymorphism in prolactin gene and milk production in cattle: A Meta-analysis

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SUMMARY

Numerous studies have examined the association between prolactin gene polymorphisms with different traits of milk production in cow, often with conflicting results. This study was performed to investigate the association between *PRL/Rsa I* polymorphism and milk production by meta-analysis of various published research results. In this meta-analysis the Metafor package of R software was used to analyze the data. Based on these results, the overall effect of this gene on milk production is 0.533 with a 95% confidence interval between 0.179 to 0.887 and animals with AA genotype have higher production than animals with BB genotype (P <0.01). Subgroup analysis revealed that this difference in the function of genotypes is related to non-Holstein cows, so that cows with AA genotypes are better than BB genotype (0.66, CI 0.113 1.119, I^2=80.04%), while this difference is not significant for Holstein cows (0.37, CI -0.035 0.779, I^2=41.08%). It was also found that in additive, dominance, codominance, and recessive models when Holstein cows were studied, the difference in animal performance with different *PRL/Rsa I* genotypes was not significant. Based on these results, no association was found between *PRL/Rsa I* polymorphism with fat percentage and milk protein percentage in the studied populations. It is suggested that instead of focusing on variants on this gene as direct markers for the selection of dairy cows, the effect of this gene in combination with other genes in the framework of genomic selection should be considered.

KEY WORDS

Genetic model, milk traits, *PRL* gene, systematic review.

INTRODUCTION

Genetic selection of livestock based on traits to improve production and reproduction performance is a time-consuming process that is only possible for livestock with production records. Therefore, a suitable solution to improve these traits is to search for molecular markers in or around genes that are directly or indirectly involved in milk production or reproductive function to use genomic information along with phenotypic records for accurate estimation of breeding values. In dairy cows, one of the most important goals of selection is to increase both milk productivity and composition. Through molecular techniques and determining the basic genes affecting production traits in livestock, the efficiency of breeding programs can be enhanced[1].

Candidate genes for a particular trait are sequenced genes whose biological activity is known. Based on the candidate gene hypothesis, much of the genetic diversity of quantitative traits is due to the diversity of functions of genes directly involved in physiology or production. Single nucleotide polymorphisms (SNPs) are believed to occur in some genes and may affect the gene product or at least the DNA marker of the underlying regions of the genome[2]. For more than fifty years, many studies have been conducted on different forms of genes affecting the economically important traits of cow's milk[3]. Genes encoding milk proteins and hormones are good candidates for locally effective trait markers in milk production due to their crucial biological functions. Among various hormones regulating the milk production process in cattle, prolactin is of great importance[4]. Prolactin (*PRL*) gene (ENSBTAG00000015274.4) is a polypeptide hormone synthesised and secreted from the specialised cells of the anterior pituitary gland. It is essential for the initiation and maintenance of lactation, and it is also mainly responsible for the synthesis of milk proteins, lactose, lipids, and all other major components of milk[1,5,6]. In mammals, prolactin is responsible for the onset and maintenance of lactation, the growth of the mammary glands, and lactogenesis. Given the effects described above, it can be hypothesized that the DNA variants in this gene could be used as potential genetic markers for milk yield in cattle[7]. The bovine *PRL* gene is located on chromosome 23 and comprises five exons and four introns, spanning a 10

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kb genomic segment and it encodes a 199 amino acid mature protein in BTA23[8]. It has a molecular weight of approximately 22-kDa. It is a single-chain polypeptide of 198 amino acids and involved in many endocrine activities[9]. Many researchers reported that the *PRL* gene is highly polymorphic and had an association with milk production traits[10]. A non-synonymous A G transition mutation in exon-3 of bovine *PRL* gene genotyped by Rsa I restriction endonuclease enzyme, has become a prevalent genetic marker for several production and reproduction traits[11]. Two allelic variants (A and B) have been distinguished at the DNA level, based on *Rsa I* polymorphism. The *PRL/Rsa1* locus had a significant effect on milk production and fat percentage in dairy cattle[10,12,13,14].

Based on the statistical and mathematical principles, meta-analysis is a systematic review of quantitative research. Integrating the results from different studies with a single subject, compared to the findings of an individual research, allows for a more accurate and reliable estimation. The challenge for biologists is to discover ways to analyze scattered data to help them understand the complex dynamic system of life. Meta-analysis is a statistical method for integrating data from different surveys with related hypotheses, which is a valuable way to increase the analytical power of individual surveys. In this regard, new and powerful tools have been developed for the analysis of expressive data.

One of the most important goals of meta-analysis studies is to provide an accurate and valid result, by increasing the sample size due to the combination of different studies and hence reducing the confidence interval of these measurements and solving problems resulting from controversial results of previous studies[15].

The term meta-analysis is used to refer to the statistical analysis of a large set of results from individual studies to integrate findings[16]. Like any statistical method, meta-analysis has its pros and cons but is now one of the standard tools for providing a clear, objective and repetitive summary of research findings in the social sciences, medicine, education, and other fields[17]. According to the many studies that have been conducted on the prolactin gene mutation (*PRL/Rsa I*) on different breeds of cattle, which have led to different and sometimes contradictory results, it seems that it is necessary to summarize the effect of this mutation on the production of milk, fat, and protein based on more data. Therefore, this meta-analyze was performed to investigate the association between polymorphism studies of *PRL/Rsa I* polymorphism with milk production and the dependent traits of fat percentage and protein percentage.

MATERIALS AND METHODS

Published scientific papers on the relationship between *PRL/Rsa I* polymorphism and milk production in cattle were retrieved from several databases (Web of Science, Science Direct, and Google Scholar); out of which a sample consisting of 98 original research papers published between 2000 and 2020 was selected. This number was selected by refining and finally, 21 articles were selected for the subsequent analysis. All selected articles met all three inclusion criteria including 1) examining the relationship between *PRL/Rsa I* polymorphism and milk production. 2) Investigation of three genotypes (AA, AB, and BB). 3) Existence of mean report, and standard deviation or standard error for milk production, milk fat percentage, and

milk protein percentage traits for each genotype.

All necessary information from selected articles was entered in a table. This table included information about the characteristics of the corresponding author(s), year of publication, country, breed, the number of animals tested, the number of genotypes observed, average milk production for each genotype, standard deviation from the mean and significant level of relationship. Before statistical analysis, this information was arranged according to the corresponding author to prevent errors. In the cases with no standard deviation, the standard deviation value was calculated using the following equation:

$$SE = \frac{SD}{\sqrt{n}}$$

where, SE stands for the standard error of the genotype and n represents the number of records for each genotype.

The mean standardized difference was obtained using the following equation[18]:

$$d = \frac{\bar{X}1 - \bar{X}2}{SD_{pooled}}$$

In this equation, 1 and 2 are the means of the two groups compared and SD_{pooled} is the cumulative standard deviation of both groups obtained from the following equation:

$$SD_{pooled} = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}}$$

where, n_1 and n_2 are the sample size and S_1 and S_2 are the standard deviations in each group.

Statistical Analysis:

Metafor statistical package of R software was used to analyze the data in this meta-analysis. The collected data were divided into three groups based on the breed of cattle, including Holstein, other breeds (Jersey, Gir, Conkraj, Sahiwal, and Dioni) and the combination of these two groups, the results of this analysis are presented in Table 4. Four different genetic models (AA vs BB additive model, AA + BB vs AB co-dominant model, AA + AB vs BB dominance model, and AA vs AB + BB recessive model) were used to evaluate the relationship between *PRL/Rsa I* polymorphism and milk production.

Heterogeneity between studies was calculated using the I^2 parameter whose value varies between zero and 100; that is I^2 less than 50 indicates low heterogeneity and I^2 above 50 indicates high heterogeneity. A random model should be used for the analysis of studies with high heterogeneity[19].

The genotypic and allelic frequencies of *PRL/Rsa I* polymorphisms in the various studies used in this survey are shown in Table 1.

RESULTS

The results of the meta-analysis for the association between *PRL/Rsa I* polymorphism and milk production based on four genetic models including additive, dominant, co-dominant, and recessive are presented in Tables 2-4.

The relationship between polymorphism of *PRL/Rsa I* and milk production: The results of the heterogeneity test based on I^2 and the number of studies used in Meta-analysis show that almost all models have a high heterogeneity, even using a random model. Although animal grouping into two groups of Hol-

Table 1 - Allelic and genotypic frequencies of *PRL/Rsa I* polymorphism in different studies for meta-analysis.

No	Year	Breed	Total	Genotype Frequencies						Allele Frequencies					
			Number	nAA	%AA	nAB	%AB	nBB	%BB	A	%A	B	%B	χ^2	P
1	2007	Holstein	72	36	0.50	30	0.42	6	0.08	51	0.71	21	0.29	0.0002	P>0.05
2	2007	Holstein	98	59	0.60	38	0.39	1	0.01	78	0.80	20	0.20	0.0996	P>0.05
3	2002	Holstein	900	657	0.73	235	0.26	8	0.01	775	0.86	125	0.14	0.0235	P>0.05
4	2002	Holstein	600	426	0.71	166	0.28	8	0.01	509	0.85	91	0.15	0.0168	P>0.05
5	2002	Holstein	366	255	0.70	104	0.28	7	0.02	307	0.84	59	0.16	0.0075	P>0.05
6	2005	Jersey	185	17	0.09	80	0.43	88	0.48	57	0.31	128	0.69	0.0002	P>0.05
7	2005	Jersey	147	13	0.09	63	0.43	71	0.48	45	0.31	102	0.69	0.0002	P>0.05
8	2005	Jersey	91	6	0.07	37	0.41	48	0.53	25	0.27	66	0.73	0.0010	P>0.05
9	2005	Holstein	242	172	0.71	69	0.29	1	0.01	207	0.86	35	0.14	0.0578	P>0.05
10	2005	Holstein	162	117	0.72	44	0.27	1	0.01	139	0.86	23	0.14	0.0399	P>0.05
11	2005	Holstein	94	69	0.73	24	0.26	1	0.01	81	0.86	13	0.14	0.0155	P>0.05
12	2008	Holstein	720	133	0.18	572	0.79	15	0.02	419	0.58	301	0.42	0.6610	P>0.05
13	2019	Holstein	150	108	0.72	41	0.27	1	0.01	129	0.86	21	0.14	0.0385	P>0.05
14	2017	Gir	200	9	0.05	166	0.83	25	0.13	92	0.46	108	0.54	0.5678	P>0.05
15	2017	Kankrej	100	13	0.13	70	0.70	17	0.17	48	0.48	25	0.25	0.2131	P>0.05
16	2008	Kankrej	51	17	0.33	18	0.35	16	0.31	26	0.51	25	0.49	0.1213	P>0.05
17	2008	Kankrej	51	17	0.33	18	0.35	16	0.31	26	0.51	25	0.49	0.1213	P>0.05
18	2088	Kankrej	51	17	0.33	18	0.35	16	0.31	26	0.51	25	0.49	0.1213	P>0.05
19	2008	Kankrej	51	17	0.33	18	0.35	16	0.31	26	0.51	25	0.49	0.1213	P>0.05
20	2015	Sahiwal	126	75	0.60	39	0.31	12	0.10	95	0.75	31	0.25	0.0734	P>0.05
21	2012	Dioni	72	7	0.10	42	0.58	23	0.32	28	0.39	44	0.61	0.0567	P>0.05

stein cows and other breeds reduces the level of heterogeneity, in most cases heterogeneity is still high (Table 2).
An additive model: Comparison of genotypes in this model (AA genotype versus BB genotype) indicates that the average yield of milk production in animals with AA, and BB genotypes are 5999.12, and 3907.17 liters; respectively. Supplementary Table 1 presents the results of the meta-analysis with an additive model for the relationship between the *PRL* gene, and milk production. Based on these results, the overall effect of this gene on milk production is 0.533 with a 95% confidence interval between 0.179 to 0.887 and animals with AA genotype have higher production than animals with BB genotype (P <0.01). But based on I^2, there is significant heterogeneity between observations and hence, the results should be evaluated more carefully. With separate analysis for Holstein cows and non-Holstein cows, it is determined that this difference in the function of genotypes is related to non-Holstein cows so that cows with AA genotypes are better than BB genotype (0.66, CI 0.113 1.119, I^2=80.04%) While this difference is not significant for Holstein cows (0.37, CI -0.035 0.779, I^2=41.08%).
Dominant model: In this model, animals with AA + AB genotype were compared with animals with BB genotype of *PRL/Rsa I* polymorphism. Animals in the AA + AB genotypic group with an average of 5914.60 liters have a better performance than the animals of the BB genotype group with an average of 3097.17. However, the results of the meta-analysis show no significant difference between the two groups, the estimation of the dominant model effect was 0.043 (95% con-

fidence interval between 0.303 and 0.389). But in this model, there was also heterogeneity between different studies (I^2 = 86.25) and of course a very low number of animals with BB genotype can also be the reason for the lack of significant differences in the model. When the analysis was performed with a random model, the performance difference was significant (P <0.01).
Co-dominant model: In this model, animals with AB genotype were compared with animals with AA + BB genotype. Animals in AA + BB, and AB genotypic groups showed an average of 5309.51 and 5224.04 litter, respectively. But there was no significant difference. A significant difference was observed between two genotypic groups when the analysis was performed on breeds other than Holstein (P <0.05).
Recessive model: In this model, animals with AA genotype were compared with animals with AB + BB genotype of *PRL/Rsa I* polymorphism. AA genotypic group animals with an average of 5337.40 liters showed better performance than the animals of the AB + BB genotype with an average of 5228 liters, but the difference was not significant. A significant difference was observed when the analysis was performed on breeds other than Holstein (P <0.05).
Relationship between *PRL/Rsa I* **polymorphism** and milk fat percentage:
The results of the heterogeneity test based on I^2 and the number of studies used in the meta-analysis show that heterogeneity levels are high in dominant and codominant, and moderate in additive, and recessive models.

Table 2 - Estimate of *PRL/Rsa I* polymorphism effect with a different model on the milk yield.

Model	Number	Estimate	SMD[1]	Ci.l	Ci.u	p-value	I[2]
Additive AA vs BB	3783	0.533	0.181	0.179	0.887	0.003	70.900
Random	3783	0.093	0.325	0.544-	0.730	0.776	61.250
Holstein	2658	0.372	0.207	0.035-	0.779	0.073	41.080
Other breeds	1125	0.656	0.277	0.113	1.119	0.018	80.040
Dominant AA+AB vs BB	3783	0.043	0.176	0.303-	0.389	0.808	86.250
Random	3783	-0.828	0.339	-1.493	-0.163	0.015	81.500
Holstein	2658	0.634	0.332	-0.016	1.284	0.050	77.070
Other breeds	1125	-0.187	0.167	-0.514	0.139	0.261	81.500
Co-dominant AA+BB vs AB	4529	0.259	0.184	-0.101	0.618	0.159	95.940
Random	4529	0.469	0.363	-0.243	1.181	0.197	95.770
Holstein	3404	0.054	0.256	-0.447	0.555	0.834	97.150
Other breeds	1125	0.520	0.248	0.034	1.006	0.036	91.570
Recessive AA vs AB+BB	4529	0.283	0.171	-0.053	0.618	0.099	94.310
Random	4529	0.473	0.338	-0.189	1.134	0.161	95.770
Holstein	3404	0.089	0.227	-0.356	0.534	0.696	96.390
Other breeds	1125	0.564	0.241	0.091	1.037	0.019	81.080

[1]Standardized mean difference

Based on the results presented in Table 3, the overall effect of this gene on milk fat percentage is -0.17 with confidence intervals of 95% between -0.73 and 0.38 and animals with BB genotype have a higher fat percentage of animals with AA genotype, although this difference is not statistically significant. In random models and separate analyzes for Holstein cows and other breeds, there was no significant difference in the effect of *PRL/Rsa I* polymorphism on milk fat percentage.

Additive model: A comparison of genotypes in this model (AA genotype versus BB genotype) shows that the average percentage of milk fat in animals with AA genotype and BB genotype were 4.11 and 4.97%; respectively.

Dominant model: In this model, the percentage of milk fat in cows with BB genotype was compared with that of cows with AA + AB genotype. the results revealed that BB genotypic with an average of 4.97% have better performance than animals of the genotype AA + AB with an average of 4.11%. The results of the meta-analysis show no significant difference between the two groups. The estimation of the dominant model was 0.038 (95% confidence interval between 0.143 and 0.219). In this model, there was also heterogeneity between the population of different studies ($I^2 = 45.74$). Fewer number of animals with BB

genotype can explain the lack of significant differences in the model. When the analysis was done with a random model, there was no significant difference in the percentage of milk fat between the groups.

Co-dominant model: In this model, Animals with AA + BB genotypic group with an average of 4.17% compared to animals with genotype AB with an average of 4.11 had better performance, but no significant difference was observed.

Recessive model: In this model, animals with AA genotype were compared with animals with AB + BB genotype. AA, and AB+BB genotype groups have an average of 4.11 and 4.24, respectively, but there was no significant difference.

The relationship between polymorphism of *PRL/Rsa I* and milk protein percentage:

The results of the heterogeneity test based on I^2 and the number of studies used in the meta-analysis show that heterogeneity levels were high in co-dominance and dominance models, but suitable homogeneity was observed in additive and recessive models.

Additive model: A comparison of genotypes in this model (AA genotype versus BB genotype) indicates that the average percentage of milk protein in animals with AA and BB genotypes

Table 3 - Estimate of *PRL/Rsa I* polymorphism effect with a different model on the milk fat percentage.

Model	Number	Estimate	SMD[1]	Ci.l	Ci.u	p-value	I[2]
Additive AA vs BB	5113	-0.173	0.284	-0.729	0.383	0.542	91.67
Random	5113	0.48	0.862	-1.21	2.17	0.578	83.94
Holstein	4205	-0.091	0.241	-0.563	0.382	0.706	81.33
Other breeds	908	-0.326	0.786	-1.866	1.213	0.678	96.32
Co-dominant AA+BB vs AB	5113	-0.008	0.044	-0.095	0.078	0.85	37.4
Random	5113	-0.002	0.054	-0.104	0.108	0.967	44.1
Holstein	4205	0.001	0.046	-0.089	0.091	0.978	29.63
Other breeds	908	-0.035	0.139	-0.307	0.237	0.8	64.69
Dominant AA+AB vs BB	5113	0.038	0.092	-0.143	0.219	0.682	45.74
Random	5113	-0.012	0.125	-0.258	0.233	0.922	46.47
Holstein	4205	-0.014	0.108	-0.225	0.198	0.899	21.09
Other breeds	908	0.116	0.196	-0.268	0.499	0.554	77.12
Recessive AA vs AB+BB	5113	0.002	0.042	-0.081	0.085	0.965	23.36
Random	5113	0.003	0.047	-0.089	0.095	0.998	27.22
Holstein	4205	0	0.044	-0.086	0.086	0.999	23.33
Other breeds	908	0.02	0.153	-0.279	0.319	0.896	41.2

[1]Standardized mean difference

were 3.25 and 3.67; respectively. The results of meta-analysis with the additive model for the relationship between the *PRL* gene and the percentage of milk protein are presented in Table 2. According to the results, the overall effect of this gene on the protein percentage of milk is 0.569 with a 95% confidence interval between -0.27 and 1.14. The animals with BB genotype have a higher performance compared to those with AA genotype, although this difference isn't significant. In random models and separate analyzes for Holstein cows and other breeds, there was no significant difference in the effect of *PRL/Rsa* I polymorphism on the percentage of milk protein.

Dominance Model: In this model, the percentage of milk fat compared, animals with BB genotype versus animals with AA + AB gene of *PRL/Rsa I*. there is a significant difference between the animals in genotypic group AA + AB with an average of 3.32% compared to the animals of the genotype BB (P<0.05). Although in the analysis of this model, the effect of the dominant model was 0.196 (95% confidence interval between 0.002 and 0.398). In this model there was also heterogeneity between different studies (I^2 = 42.93). When the analysis was repeated with a random model, there was no significant difference.

Co-dominant model: This model compares AA + BB, and AB genotypes. There was no significant difference between animals in the AA + BB genotype group with an average of 3.28% protein milk and the genotypic group AB with an average of 3.31% protein milk.

Recessive model: In this model, animals with AA genotype were compared with animals with AB + BB genotype of *PRL/Rsa I* gene. Animals in the AA genotypic group with an average of 3.25% showed lower yield compared to the animals of the AB + BB genotype group with an average of 3.27%, but the difference was not significant.

Most studies on the correlation between the polymorphisms of genes and different production traits in certain breeds are constrained to a limited number and in certain areas. And sometimes the abundance of some genotypes is slightly observed, which accompanies the general conclusion about the relationship between the multiplexing of the study gene and traits with doubt. It has been suggested that in these cases, with cumulative data and with meta-analysis assistance, comparisons can be compared with the larger number of samples in such a way that the results may be more comprehensive[20].

DISCUSSION

Most studies[1,13,21,22,23] have shown that the A allele and AA genotype had the highest frequency for the *PRL* gene in most of cattle breeds. Although studies have shown that the BB genotype in the Holstein breed is rare, the BB genotype has a higher abundance in other breeds[1,14,24,25]. These findings may reflect the negative effect of Holstein cow selection criteria on the frequency of the B allele, while other breeds, especially local breeds, show a higher frequency of allele B and the BB genotype.

Contrary to the clear effect of selection on reducing the frequency of B alleles and BB genotype, in the effect of this allele and genotype on milk production, regardless of the breed of cows, has been suggested in various results. While others[14,21] showed that Holstein cows with BB genotype produced more milk compared to two AA and AB genotypes; in other research[1,2,26,27,28] AA genotype has been reported as the main cause of the increased milk production performance and milk fat and in some studies there is no significant difference between *PRL/Rsa I* genotypes for milk production or other traits[7,23,25]. In various studies[29,30,31] while the AA genotype had a better performance for milk production (P <0.05), no difference has been reported between *PRL/Rsa I* polymorphism with fat and protein percentage in the Holstein breed of different parts of the world. A similar result was obtained for Russian Simmental cows[23].

Some other studies[13,32] have maintained that Holstein cows in Poland with BB genotype had the lowest milk and milk fat percentage compared to two other genotypes of *PRL/Rsa I*; this result may be attributed to the negative correlation between milk production and fat percentage. While the percentage of protein does not show a significant difference between the three genotypes. In a study[1], it was observed that Holstein cows in Poland AA have the highest milk production, while the BB genotype had the lowest percentage of protein.

In previous studies, animal with BB genotype were associated with higher annual milk yield while there were not significant differences for milk fat and milk protein percentage, the same results were observed in comparison with *PRL/Rsa I* genotypes for indigenous breeds Gir, and Kankrej [24]. In other studies[33], genotyped animals for the *PRL/Rsa I* did not have any significant difference for milk production traits, milk fat percentage,

Table 4 - Estimate of *PRL/Rsa I* polymorphism effect with different model on the milk protein percentage.

Model	Number	Estimate	SMD¹	Ci.l	Ci.u	p-value	I²
Additive AA vs BB	4786	0.569	0.43	-0.274	1.413	0.186	95.93
Random	4786	0.75	0.496	-0.221	1.722	0.13	95.66
Holstein	4178	0.75	0.522	-0.274	1.744	0.151	95.81
Other breeds	608	-0.052	0.618	-1.264	1.16	0.933	92.6
Co-dominant AA+BB vs AB	4786	0.048	0.06	-0.07	0.165	0.428	62.25
Random	4786	0.09	0.063	-0.033	0.214	0.152	57.28
Holstein	4178	0.085	0.055	-0.022	0.191	0.121	46.2
Other breeds	608	-0.156	0.222	-0.599	0.272	0.462	78.72
Dominant AA+AB vs BB	4786	0.196	0.099	0.002	0.398	0.048	42.93
Random	4786	0.212	0.125	-0.032	0.456	0.089	41.98
Holstein	4178	0.203	0.135	-0.062	0.467	0.133	45.58
Other breeds	608	0.126	0.088	-0.046	0.299	0.152	59
Recessive AA vs AB+BB	4786	0.184	0.12	-0.052	0.42	0.126	90.27
Random	4786	0.226	0.137	-0.042	0.494	0.099	91.07
Holstein	4178	0.227	0.142	-0.052	0.505	0.111	92.63
Other breeds	608	0.029	0.198	-0.36	0.417	0.885	52.96

¹Standardized mean difference

and milk protein percentage.

CONCLUSION

In this study, the relationship between *PRL/Rsa I* polymorphism and milk production, milk fat percentage, and milk protein percentage were investigated according to different models. The results of this meta-analysis showed that in order to study the relationship between prolactin gene polymorphism and milk production, an additive model is more suitable than other models. While there is no correlation between prolactin gene polymorphism and fat percentage and milk protein percentage. By comparing the frequencies of the *PRL/Rsa I* in Holstein cows in different parts of the world with other breeds of dairy cows, it can be found that the selection for milk production increases the AA genotype and, as compared to other milk breed cattle, with lower or local milk production. It is due to pressure selection on this gene. According to these results, *PRL/Rsa I* is not a very important gene affecting milk production, fat percentage, and protein percent of milk. Therefor, instead of focusing on this gene as a candidate gene for the milk yield and milk composition in dairy cattle, the effect of this gene in combination with other genes, in the framework of genomic selection should be considered.

Disclosures
The authors declare no real or perceived conflicts of interest.

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