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Association of TLR6 gene polymorphisms with zootechnical parameters in Holstein cattle in northern Antioquia

Asociación de polimorfismos del gen TLR6 con parámetros zootécnicos en ganado Holstein del norte de Antioquia

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ABSTRACT

Specialized dairy systems are one of the most important lines in the agricultural sector in Colombia and the world. Currently there are strategies, both of environmental management and animal breeding, seeking to optimize the production. TLR6 gene, as many others related to immunity, is proving to be a useful candidate for programs of Marker Assisted Selection (MAS). Four hundred thirty two Holstein cows from 4 municipalities of northern Antioquia were genotyped using PCR-RFLP for C1859A and A1980G polymorphism of TLR6 gene. Allelic frequencies of 57.87% were found for C allele and 42.13% for A allele of C1859A, and 57.99% for A allele and 42.01% for G allele of A1980G. Genotypic frequencies of C1859A were 84.26% and 15.74% for CA and CC, respectively, and no AA genotype for this polymorphism was found; phenotypic frequencies of A1980G were 25.93%, 64.12% and 9.95% for AA, GA and GG, respectively. Mixed models for repeated measures with random effect of the animal were used to determine the association between genotypes and milk yield, percentages of protein and fat, services per conception, calving interval, and SCS. Both polymorphisms were only significant (P<0.01) on the SCS feature, being C allele of C1859A and A allele of A1980G the most favorable. TLR6 gene showed an effect on quality milk trait SCS, as expected for its immunological role in the response against bacteria, and can be postulated as molecular marker for selection against infectious diseases of importance in animal production, such as mastitis.

Keywords:
Allele
Cattle
Genotype
Immunity
PCR
RFLP

RESUMEN

Los sistemas de lechería especializada son uno de los renglones más importantes en el sector agropecuario de Colombia y del mundo. Actualmente existen estrategias, tanto de manejo ambiental como de mejoramiento animal, que buscan optimizar la producción. El gen TLR6, como muchos relacionados con la inmunidad, está demostrando ser un candidato útil para los programas de selección asistida por marcadores moleculares (MAS). Mediante PCR-RFLP, se genotipificaron 432 vacas Holstein de 4 municipios del Norte de Antioquia para los polimorfismos C1859A y A1980G del gen TLR6. Se encontraron frecuencias alélicas de 57,87% para el alelo C y 42,13% para el alelo A de C1859A, y 57,99% para el alelo A y 42,01% para el alelo G de A1980G. Las frecuencias genotípicas de C1859A fueron 84,26% y 15,74% para CA y CC, respectivamente, y no se halló el genotipo AA para este polimorfismo; las frecuencias fenotípicas de A1980G fueron 25,93%, 64,12% y 9,95% para AA, GA y GG, respectivamente. Se utilizaron modelos mixtos de repeticiones con efecto aleatorio del animal para determinar asociación entre los genotipos y las características zootécnicas producción de leche, porcentajes de proteína y grasa, servicios por concepción, IEP y SCS. Ambos polimorfismos solo tuvieron efecto significativo (P<0,01) sobre la característica SCS, siendo el alelo C de C1859A y el alelo A de A1980G los más favorables. El gen TLR6, mostró un efecto sobre el parámetro SCS de calidad de leche, como se espera por su role inmunológico en la respuesta contra bacterias, y puede ser postulado como marcador molecular para la selección en contra de enfermedades infecciosas de importancia en producción animal, como la mastitis.

Palabras clave:
Alelo
Bovinos
Genotipo
Inmunidad
PCR
RFLP

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The dairy sector is one of the leading in agricultural production in the world, being milk the main livestock product in most countries where it is produced (Hemme *et al*., 2012; Knips, 2005). In Colombia, this sector covers 6.4% of dairy herds, producing up to 6716 million liters in 2014 (FEDEGAN, 2014, 2015). In specialized dairy, predominantly in the department of Antioquia, the most common breed is Holstein, which extensive information has been collected in recent years due to record management (Jaramillo and Areiza, 2012).

To optimize production and reproduction, especially in specialized dairy, modern techniques and technologies have been adopted, particularly regarding the selection of the animals and their traits. One of these techniques is Marker-Assisted Selection (MAS), which uses gene variations that confer advantage as a selection criterion (Singh *et al*., 2014). One of the candidate genes to be used as a molecular marker is TLR6, which is a receptor of antigen-presenting cells that can form complexes with TLR2 (Takeda and Akira, 2005), and together may recognize different bacterial antigens, especially zymosan, lipopeptides and lipopolysaccharide (Kang *et al.,* 2011). TLR receptors, including TLR6, have been linked with reproduction and immunological traits (Kannaki *et al*., 2011) as resistance to bacterial diseases like tuberculosis (Sun *et al*., 2012; Song *et al*., 2014) or mastitis (Wang *et al*., 2007; Russell *et al*., 2011) and quality milk traits like SCS (Sharma *et al*., 2006; Chu *et al*., 2009; Zhang *et al*., 2009; Beecher *et al*., 2010).

Every day, the demand for dairy products in our country grows and zootechnical ways for supply it are needed. Due to TLR's role in immune response and health of the animal, and due to only Ramírez *et al*. (2014) have studied TLR receptors and their effect on cattle traits in Colombia, it is important to know, first, the circulating TLR6 polymorphisms genotypes in our cattle populations, and second, if there is a significant effect of the gene on zootechnical traits; all of this for a better designing of breeding and genetic improvement programs in dairy herds.

The aim of this study was to associate C1859A and A1980G polymorphisms of TLR6 gene to productive and reproductive parameters of Holstein cattle in northern Antioquia, to determine favorable alleles for these parameters.

MATERIALS AND METHODS

Place and experimental units

Blood samples from 432 Holstein cows of municipalities of the northern subregion of Antioquia (Colombia) Belmira, San Pedro de los Milagros, and Entrerríos, and from the small town of San Felix, municipality of Bello, were taken. These cows belong to the project "Genetic Evaluation and Dairy Control Program" from the BIOGEM group of the Universidad Nacional de Colombia, Medellín Campus, and Colanta LTDA.

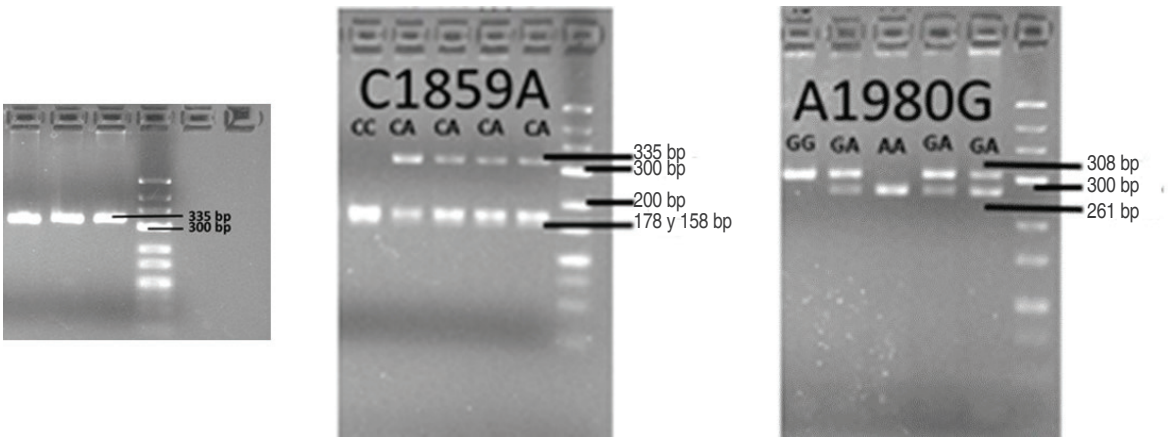
Blood samples were taken by puncture in the middle coccygeal vein and were collected in BD Vacutainer tubes with purple system (EDTA as anticoagulant). They were kept under refrigeration until processing (extraction of DNA), in the Laboratory of Basic Animal Science at the Universidad Nacional de Colombia, Medellín Campus.

DNA extraction and genotyping by PCR-RFLP

DNA extraction was carried out by salting out procedure (Miller *et al*., 1988). For amplification by PCR and genotyping by RFLP for C1859A and A1980G polymorphisms, primers and protocols described by Song *et al.* (2014), which amplify from position 1689 to 2023 of exon 3 of TLR6 gene, according to the sequence NM_001001159 reported in GenBank, were used. Primers used were F: 5'-GGTATCTTTAGCAGCCTTTCCATAC-3' and R: 5'-GTCACAGCAACAGCCAGCA-3' and PCR protocol was done in a total volume of 25 µL containing 0,4 mM of each dNTP, 1 µM of each primer, 2.5 µL of 10X buffer, 2.5 µL of 25 mM $MgCl_2$, ultrapure water, 1 unit of Taq DNA polymerase and from 100 to 200 ng of genomic bovine DNA. Amplification was done with the following program of thermocycling: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 40 s, alignment at 65 °C for 30 s, extension at 72 °C for 30 s, followed by a final extension at 72 °C for 7 min. The expected bands are 335 bp.

Digestion with BstXI enzymes (for C1859A polymorphism) and BclI (for A1980G polymorphism) was performed in a final volume of 10 µL, including 3 µL of the PCR product, 5 units of restriction enzyme, 1 µL of the corresponding buffer and ultrapure water. Reactions were incubated at 37 °C for 16 hours and visualized on 3% agarose gels stained with EZ-Vision. The bands obtained by digestion with BstXI were of 335 bp for AA genotype, 335 bp/178

bp/158 bp for CA genotype and 178 bp/157 bp for CC genotype. The bands obtained by digestion with BclI are 308 bp/27 bp for GG genotype, 308 bp/261 bp/47 bp/27 bp for GA genotype and 261 bp/47 bp/27 bp for AA genotype. Bands of 47 and 27 bp are not displayed on the gel due to its size and staining resolution (Figure 1).



The last well of each electrophoretic run image corresponds to the molecular weight marker.

Figure 1. PCR and RFLP banding patterns.

Frequencies calculation and Hardy – Weinberg equilibrium

Frequencies were calculated as the proportion between alleles, genotypes or haplotypes of each type and the total of alleles, genotypes or haplotypes in the population, as Hartl described it in 2007. Hardy – Weinberg equilibrium state was calculated with allelic frequencies observed and expected in software GenAlEx 6.503 (Peakall and Smouse, 2012).

Evaluated productive and reproductive parameters

All information of the animals and of all animal lactations were compiled by the "Genetic Evaluation Program and Dairy Control" for 5 years. The parameters evaluated were total milk yield (TMY) in Kg, percentages of protein and fat in milk per lactation (PP and FP), amounts of protein and fat in milk per lactation (PA and FA) in Kg, calculated with each percentage and the total milk yield, calving interval (CI), services per conception (SxC), and somatic cell score (SCS), calculated by logarithmic transformation of somatic cell count in milk (SCC) with the formula:

$$SCS = Log2(SCC/100) + 3$$

Data were adjusted by eliminating extreme values that could alter the performance of the model; lactations under 250 days and over 450 days, TMY under 2500 kg and over 12000 kg and SxC equal to 0 were removed.

Association of polymorphisms with productive and reproductive parameters

Descriptive analysis of the evaluated parameters was made and frequency tables were used to characterize averages of the parameters according to genotype. To make the association, a mixed model with random effect of the animal and Tukey's range test was used. The models for each trait were evaluated by the Bayesian Information Criterion (BIC), which value indicates the quality of the model; while the value is lower, the model fits better. A linear regression was used to assess the effect of the substitution of each allele, and averages were evaluated. All calculations were made with SAS® software version 9.2 for Windows (SAS Institute Inc, Cary NC, USA).

The mixed model was:

$$Y_{ijklmnop} = \mu + N_i + (H_j * A_k * M_l) + G_m + L_n + P_o + E_{ijklmnop} \quad (1)$$

Being:

$Y_{ijklmnop}$: Productive or reproductive trait

μ: Population average of productive or reproductive trait

N_i: Effect of number of calvings

Contemporary group:
H_j: Effect of herd
A_k: Effect of calving year
M_l: Effect of calving month
G_m: Fixed effect of the gene (genotype)
L_n: Effect of lactation duration
P_o: Effect of the total milk yield per lactation
$E_{ijklmnop}$: Random experimental error
For TMY trait, P_o variable was not used

The linear regression model was:

$$Y_{ijk} = \beta_o + \beta_i L_i + \beta_j G_j + \beta_k P_k \qquad (2)$$

Being:
Y_{ijk}: Productive or reproductive trait
β_0: Intersection or constant
β_i, β_j, β_k: Parameters of each variable
L_i: Effect of lactation duration
G_j: Fixed effect of the alleles
P_k: Effect of the total milk yield per lactation

RESULTS AND DISCUSSION

C1859A and A1980G polymorphisms of TLR6 gene of 432 animals from the municipalities of Bello, Belmira, Entrerríos and San Pedro de los Milagros were amplified. The first polymorphism generates no amino acid change and the second causes a change from isoleucine to valine, which, although is also an apolar and hydrophobic amino acid, has lower molecular weight. The three genotypes of A1980G polymorphism were found, but for C1859A polymorphism only CA and CC genotypes were found in the analyzed population (Table 1). The most frequent alleles were C for C1859A polymorphism and A for A1980G polymorphism; the most frequent genotypes were heterozygous ones in both polymorphisms and, in the case of A1980G, GA is followed by AA genotype. C1859A/A1980G most common haplotype was the CA/GA, followed by CC/AA, CA/AA and CA/GG. By municipality CA/GA remains the most common haplotype, followed by CC/AA, except in Bello, where it is followed by CA/GG; in Belmira, CA/AA and CA/GG haplotypes are equally represented, as are CC/AA and CA/AA haplotypes in Entrerríos. AA genotype of A1980G polymorphism was the only one found with the two genotypes of C1859A polymorphism; the other two were always found with genotype CA of C1859A.

Genotypic and allelic frequencies found are similar to the only two papers reported for these polymorphisms. Song *et al*.

Table 1. Allele, genotype and haplotype frequencies by municipality.

Polymorphism			Bello	Belmira	Entrerrios	San Pedro	Total
C1859A	ALLELES	A	45	44.44	41.77	39.78	42.13
		C	55	55.54	58.23	60.22	57.87
	GENOTYPES	CA	90	88.89	83.54	79.56	84.26
		CC	10	11.11	16.46	20.44	15.74
		AA	0	0	0	0	0
A1980G	ALLELES	A	51.36	55.54	61.39	59.85	57.99
		G	48.64	44.44	38.61	40.15	42.01
	GENOTYPES	AA	15.45	14.81	32.91	28.47	25.93
		GA	71.82	81.48	56.96	62.77	64.12
		GG	12.73	3.7	10.13	8.76	9.95
HAPLOTYPES C1859A/A1980G		CA/AA	5.45	3.7	16.45	8.03	10.18
		CA/GA	71.82	81.48	56.96	62.77	64.12
		CA/GG	12.73	3.7	10.13	8.76	9.95
		CC/AA	10	11.11	1645	20.44	15.74
		CC/GA	0	0	0	0	0
		CC/GG	0	0	0	0	0

(2014) reported frequencies in Holstein cattle in China above 70% for CA genotype of C1859A polymorphism, followed by CC and AA, which was below 10%, which shows its low frequency in other populations of Holstein cattle and allows to suggest that this allele may be disappearing due to the selection processes that Holstein breed has been subjected to. For A1980G polymorphism, the most common genotype was GA, having a frequency of almost 60%. Chu *et al*. (2009) did not find AA genotype either and found allelic and genotypic frequencies in Holstein cattle from China very similar to those found in this work; reporting allele frequencies of 58.9% for C and 41.1% for A; and genotype frequencies of 17.8% for CC and 82.2% for CA. For A1980G polymorphism, allele frequencies of 54.3% for A and 45.7% for G; and genotype frequencies of 26% for AA genotype, 56.7% for GA and 17.3% for GG were found (Chu *et al*., 2009).

In Table 2 are shown the X^2 and P values of Hardy – Weinberg equilibrium state analysis. All polymorphisms in all municipalities are in disequilibrium state, except A1980G polymorphism in Belmira. As said above, the artificial selection processes the Holstein cattle suffer may be causing the frequencies of some alleles of TLR6 polymorphisms are reducing.

In Table 3, the mean and CV of the analyzed traits are shown.

Table 2. Hardy – Weinberg Equilibrium analysis.

Municipality	Locus	Degree of freedom	X^2	*P* value
Bello	C1859A	1	50.77	<0.01**
Bello	A1980G	1	12.00	<0.01**
Belmira	C1859A	1	7.96	<0.01**
Belmira	A1980G	1	1.98	0.16
San Pedro de los Milagros	C1859A	1	76.08	<0.01**
San Pedro de los Milagros	A1980G	1	10.17	<0.01**
Entrerríos	C1859A	1	97.44	<0.01**
Entrerríos	A1980G	1	27.59	<0.01**

Values with ** are very significant (*P*<0.01)

Table 3. Mean of the evaluated trait.

Trait	Mean	Standard deviation	CV
Lactation Period (days)	333.43	48.99	14.69
Total Milk Yield (kg)	5643.20	1746.28	30.94
Protein Porcentage	2.98	0.23	7.80
Protein Amount (kg)	155.53	37.97	24.41
Fat Porcentage	3.77	0.46	12.27
Fat Amount (kg)	197.59	55.32	28.00
Services per Conception	1.78	1.20	67.28
Calving Interval (days)	444.76	159.28	35.81
Somatic Cell Score	4.34	1.32	30.49

The average milk yield found in this work (5643.2 kg) is higher than that recorded by Quijano *et al*. (2011), 5140 kg per lactation; protein (2.9%) and fat percentage (3.77%) of this study are similar to those reported by this author (3.07% and 3.8% respectively), but the CI (445 days) was greater than the one they found (417

days). The Holstein Association of Colombia recorded a higher average yield of (6237 kg) and SxC (2.57). Jaramillo *et al*. (2012), reported for the so called Region 1, which covers departments known for its dairy orientation including Antioquia, protein and fat percentages of 3.07 and 3.63 respectively for 2012; nationwide, they reported 3.1% and 3.66% respectively. The SCS found in this study was considerably higher than the 3.06 reported by Quijano *et al*. (2011), probably due to the smaller sample number and the smaller number of analyzed regions in this work. Gaviria *et al*. (2014) also reported SxC of 1.66 in different municipalities of Antioquia, being more favorable the results of this study. Also they recorded in different municipalities of the northern region of Antioquia an adjusted milk yield to 305 days of 5588 kg, protein and fat percentages of 3.06 and 3.89 respectively, a SCS of 4.19, a CI of 414 days and a SxC of 1.67; these results show a better trend than those found in this work, with higher yields and lower CI and SCS (Gaviria *et al*., 2014, 2015).

The association analysis showed that none of the polymorphisms had a significant effect on the evaluated trait, except for SCS, in which the effects were significant (P<0.01), including the effect of the haplotype (Tables 4 and 5). Since from C1859A polymorphism only CA and CC

Table 4. Trait means of C1859A genotype.

Trait	P value in the model	C1859a Polymorphism			
		CA		CC	
		Average	CV	Average	CV
TMY	0.59	5482.27	31.30	5525.01	32.34
PP	0.82	3.00	8.31	2.96	7.59
PA	0.56	151.90	24.86	149.68	24.37
FP	0.99	3.75	12.56	3.70	14.17
FA	0.97	190.62	28.27	187.16	27.65
SxC	0.62	1.57	68.68	1.62	66.77
CI	0.53	84.01	37.49	452.22	34.91
SCS	<0.01 **	4.47 [a]	30.99	4.06 [b]	31.87

Total milk yield in kg: TMY, percentages of protein and fat in milk per lactation: PP and FP, amounts of protein and fat in milk per lactation in kg: PA and FA, calving interval: CI, services per conception: SxC, somatic cell score: SCS
Values with ** are very significant (P<0.01). Values with different superscripts indicate significant statistical difference

Table 5. Trait means of A1980G genotype.

Trait	P value in the model	A1980G Polymorphism					
		AA		GA		GG	
		Average	CV	Average	CV	Average	CV
TMY	0.99	5444.52	30.64	5534.17	31.49	5326.05	33.39
PP	0.76	3.00	8.03	3.00	8.34	2.97	7.96
PA	0.79	150.17	22.67	153.76	25.69	141.29	22.63
FP	0.96	3.73	14.14	3.75	12.59	3.74	10.49
FA	0.99	186.69	26.05	193.27	29.15	178.98	25.71
SxC	0.92	1.57	69.67	1.6	69.36	1.54	68.45
CI	0.32	456.37	32.58	454.47	40.15	450.21	27.74
SCS	<0.01 **	4.19 [a]	33.53	4.52 [b]	30.50	4.24 [ab]	28.82

Total milk yield in kg: TMY, percentages of protein and fat in milk per lactation: PP and FP, amounts of protein and fat in milk per lactation in kg: PA and FA, calving interval: CI, services per conception: SxC, somatic cell score: SCS
Values with ** are very significant (P<0.01). Values with different superscripts indicate significant statistical difference

genotypes were found, were these genotypes the ones that had significant differences (*P*=0.0042); in the case of A1980G polymorphism, which both homozygous genotypes were found, there was a significant difference between GA and AA genotypes (*P*=0.0143). The difference of SCS between C1859A/A1980G haplotypes, with *P*=0.0087, was among CA/GA and CC/AA (Table 6). Both statistical association and the comparison of averages between genotypes and haplotypes indicate a favorable effect of C allele of C1859A and A allele of A1980G on the SCS feature.

Table 6. Trait means of C1859A/A1980G haplotype

Trait	P value in the model	Haplotypes C1859A/A1980G							
		CA/AA		CA/GA		CA/GG		CC/AA	
		Average	CV	Average	CV	Average	CV	Average	CV
TMY	0.98	5324.92	27.78	5.534.17	31.49	5326.05	33.39	5525.01	32.34
PP	0.84	3.05	8.33	3.00	8.34	2.97	7.96	2.96	7.59
PA	0.72	150.85	20.29	153.76	25.69	141.29	22.63	149.68	24.37
PG	0.95	3.77	14.13	3.75	12.59	3.74	10.49	3.70	14.17
FA	0.99	186.05	23.82	193.27	29.15	178.98	25.71	187.16	27.65
SxC	0.93	1.46	64.37	1.6	69.36	1.54	68.45	1.62	66.77
CI	0.45	462.01	29.46	454.47	40.15	450.21	27.74	452.22	34.91
SCS	<0.01**	4.38^{ab}	35.23	4.52^{a}	30.50	4.24^{ab}	28.82	4.06^{b}	31.87

Total milk yield in kg: TMY, percentages of protein and fat in milk per lactation: PP and FP, amounts of protein and fat in milk per lactation in kg: PA and FA, calving interval: CI, services per conception: SxC, somatic cell score: SCS
Values with ** are very significant (*P*<0.01). Values with different superscripts indicate significant statistical difference

In the work of Chu *et al*. (2009) it was found that A allele of A1980G polymorphism confers resistance to mastitis, giving SCS values significantly lower than the G allele, but found no association between C1859A polymorphism and mastitis. Chu *et al*. (2009) also associated other polymorphisms of TLR6, T853A and G855A, with mastitis, being T and G alleles, respectively, which confer resistance. In addition, Song *et al*. (2014) related both polymorphisms with resistance to tuberculosis, being alleles C of C1859A and G of A1980G favorable, demonstrating that these polymorphisms may be useful selection molecular markers against bacterial diseases.

In both polymorphisms, homozygous genotypes were more favorable than heterozygous genotypes, that is, they had lower SCS values. Although genetic variability (heterozygosity) is usually desirable in immune response related genes (Penn *et al*., 2002) and usually heterozygous genotypes of these genes confer some advantage, for the so - called "hybrid vigour" or "heterosis", for these polymorphisms, homozygous genotypes are the ones that confer advantage in the SCS trait. Since SCS is usually related to clinical and subclinical mastitis and can serve as an indirect indicator of this type of disorders, a possible explanatory hypothesis offered in this work to the apparent advantage conferred by homozygosity in the studied population is based on the antibacterial function of TLR6.

It is known that several bacterial pathogens are related to mastitis, but the main reported in Colombia are *Staphylococcus aureus, Streptococcus agalactiae,* and *Streptococcus dysgalactiae*, and its prevalence is always the highest but it can be sectioned and depends on the region (Calderón and Rodriguez, 2008; Ramírez *et al*., 2011; Rangel *et al*., 2011; Trujillo *et al*., 2011). Since this 3 species of bacteria are the main cause of mastitis in Colombia, and bacteria of this same genus are highly related, it may be the case that a single allele of immunity is favorable or effective against all of them and genetic variability seems not so necessary. Similarly, as homozygous individuals for this favorable allele can produce more protein product of the same type, and if this product is the most effective then are these individuals who have the advantage over the heterozygous individuals.

A contrasting example of what was found in this work is what Chu *et al*. reported in 2009, who found that the

more favorable allele of A1980G was G, suggesting that the effect of this allele is more effective against pathogenic microbiota own of Chinese Holstein cattle, which is probably different from the pathogenic microbiota in Colombia; on the other hand, they found significant differences among all genotypes, being GG the better, followed by GA and this followed by AA, which does not match with the findings of the present study. More studies are needed then, where the number of herds, number of animals and data per animal are higher and allow more reliability and more concrete results that match or refute these studies.

The analysis of allelic substitution to establish the trait changes with each change of allele only yielded significant effect on the SCS (Table 7). For C1859A polymorphism changing each C for A is unfavorable for TMY, SXC, CI and SCS, while changing each A for G in A1980G polymorphism is unfavorable for PP, PA and SCS. The change from CC to AA in the C1859A polymorphism can generate an increase up to 0.74 and the change from AA to GG in A1980G polymorphism can generate an increase up to 0.22 in SCS.

Table 7. Changes in traits with each allelic substitution.

Trait	Polymorphisms	
	C1859A C>A	A1980G A>G
TMY	-23.21	23.47
PP	0.044	-0.001
PA	2.42	-0.56
FP	0.04	0.008
FA	3.2	0.99
SxC	0.04	-0.001
CI	2.39	-3.59
SCS*	0.37	0.11

Total milk yield in kg: TMY, percentages of protein and fat in milk per lactation: PP and FP, amounts of protein and fat in milk per lactation in kg: PA and FA, calving interval: CI, services per conception: SxC, somatic cell score: SCS. Values with * are significant (P<0.05)

CONCLUSIONS

Allelic and genotypic frequencies of C1859A and A1980G polymorphisms of TLR6 gene for 432 Holstein cows from northern municipalities of Antioquia were determined. The most common alleles were C for the first polymorphism and A for the second, being heterozygous ones the most common genotypes; although analyzing allelic frequencies, not allele fixation is shown, it is possible that A allele of C1859A may be suffering a process of negative selection in improved Holstein cattle, since no AA genotype was found in the population, which is concordant with reports made by other authors. The only significant effect of polymorphisms was found on the SCS trait, expected result because this gene is involved in the immune response against bacterial pathogens; C allele of C1859A and A allele of A1980G were the most favorable alleles. The results were not completely conclusive because the heterozygous genotypes were the worst performers, contrary to expectations, especially in immune-related genes. Probably homozygosity is favorable due to the relatively narrow spectrum of main mastitis pathogens. More studies that better characterize the genotype of the population of Holstein cattle in dairy municipalities of Antioquia are needed and for they continue to design better selection strategies, especially when the goal is to improve SCS without altering or changing other traits.

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