Genome-wide association studies for tick resistance in *Bos taurus* × *Bos indicus* crossbred cattle: A deeper look into this intricate mechanism

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ABSTRACT

Rhipicephalus (Boophilus) microplus is the main cattle ectoparasite in tropical areas. Gir × Holstein crossbred cows are well adapted to different production systems in Brazil. In this context, we performed genome-wide association study (GWAS) and post-GWAS analyses for *R. microplus* resistance in an experimental Gir × Holstein F₂ population. Single nucleotide polymorphisms (SNP) identified in GWAS were used to build gene networks and to investigate the breed of origin for its alleles. Tick artificial infestations were performed during the dry and rainy seasons. Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA) and single-step BLUP procedure was used for GWAS. Post-GWAS analyses were performed by gene ontology terms enrichment and gene transcription factors networks, generated from enriched transcription factors, identified from the promoter sequences of selected gene sets. The genetic origin of marker alleles in the F₂ population was assigned using the breed of origin of alleles approach. Heritability estimates for tick counts were 0.40 ± 0.11 in the rainy season and 0.54 ± 0.11 in the dry season. The top ten 0.5-Mbp windows with the highest percentage of genetic variance explained by SNP markers were found in chromosomes 10 and 23 for both the dry and rainy seasons. Gene network analyses allowed the identification of genes involved with biological processes relevant to immune system functions (*TREM1*, *TREM2*, and *CD83*). Gene-transcription factors network allowed the identification of genes involved with immune functions (*MYO5A*, *TREML1*, and *PRSS16*). In resistant animals, the average proportion of animals

showing significant SNPs with paternal and maternal alleles originated from Gir breed was 44.8% whereas the proportion of animals with both paternal and maternal alleles originated from Holstein breed was 11.3%. Susceptible animals showing both paternal and maternal alleles originated from Holstein breed represented 44.6% on average, whereas both paternal and maternal alleles originated from Gir breed animals represented 9.3%. This study allowed us to identify candidate genes for tick resistance in Gir × Holstein crossbreds in both rainy and dry seasons. According to the origin of alleles analysis, we found that most animals classified as resistant showed 2 alleles from Gir breed, while the susceptible ones showed alleles from Holstein. Based on these results, the identified genes may be thoroughly investigated in additional experiments aiming to validate their effects on tick resistance phenotype in cattle. **Key words:** breed of origin, gene network, genetic variance, Gir × Holstein crossbred

INTRODUCTION

Among the parasites that affect cattle, ticks are the main cause of economic losses in tropical and subtropical regions around the world (Molento et al., 2013). The bovine tick, *Rhipicephalus (Boophilus) microplus*, promotes several losses in bovine production due to weight loss, behavioral changes, secondary skin infections, and transmission of viral, bacterial, and protozoa pathogens that can be fatal to the host (Léger et al., 2013).

Breeds of *Bos indicus* are genetically more resistant to ticks than *Bos taurus* due the presence of naturally selected genes throughout their evolutionary process (Ibelli et al., 2012; Ayres et al., 2015). Genetic variability for bovine tick resistance has been reported (Cardoso et al., 2014), showing potential for the improvement

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of this trait. According to Walker (2011), individual ability of cattle to acquire tick immunity is hereditary. In that work, European animals showed moderate immunological capacity (approximately 85% of the larvae died due to the animal's immune defense), whereas *Bos indicus* cattle exhibited high immune defense capacity (98% of dead larvae).

Rhipicephalus microplus tick is considered the main ectoparasite affecting cattle production in Brazil, showing high prevalence in tropical and subtropical regions (Shyma et al., 2015). Due to its large climatic diversity, the search for animals adapted to the different Brazilian regions is very important for the success of milk production. In this sense, Gir cattle (*Bos indicus*), a breed which is resistant to hot temperatures and tropical diseases, are crossed with Holstein cattle (*Bos taurus*), to generate the Girolando. Girolando is a synthetic breed that is 5/8 Holstein and 3/8 Gir by definition. This breed has shown a great adaptation throughout Brazil and accounts for 80% of the milk produced in the country (Ferro et al., 2010). The evaluation of adaptive traits is of great importance to livestock production systems because it allows the identification of the most tolerant animals to biotic and abiotic stress conditions. Subsequently, these traits can be used as selection criteria in breeding programs.

Genome-wide association studies (GWAS) have been performed to identify genomic regions associated with resistance to bovine ticks (Gasparin et al., 2007; Machado et al., 2010; Porto Neto et al., 2011). Nevertheless, information about genes underlying this trait and their breed of origin are still scarce. Post-GWAS analyses, including gene networks and gene ontology enrichment analyses, as well as the identification of relevant transcription factors can contribute to a better understanding of the molecular mechanisms involved in the complex pathogen-host interaction during *R. microplus* infestation in cattle. The identification of the origin of alleles in crossbred animals (Sevillano et al., 2016; Vandenplas et al., 2016) is also important in this context.

Thus, we aimed to perform GWAS and post-GWAS analyses for *R. microplus* resistance in cattle, using an experimental Gir × Holstein F₂ population. We identified SNP in GWAS to build gene networks and to investigate the breed of origin for its alleles.

MATERIALS AND METHODS

Experimental Population and Phenotypic Data

Data were obtained from Embrapa Dairy Cattle Research Center, located in southeastern Brazil. An

experimental population was generated and evaluated for *R. microplus* resistance as described by Machado et al. (2010). Tick artificial infestations were performed in each F₂ animal during the dry (April to September) and rainy (October to March) seasons, from 2001 to 2007. After 21 d of infestation, the number of ticks was measured in each F₂ animal. Animals were evaluated in contemporary groups aged between 10 and 14 mo. Additional traits that might affect the tick resistance evaluations were also assessed, including coat color, coat thickness, and length, density, and type of hair.

DNA Extraction and SNP Genotyping

Blood samples from parental, F₁, and F₂ populations were collected using vacuum tubes containing anticoagulant. The DNA was extracted from white blood cells using a modified phenol/chloroform method (Machado et al., 2010). Quality and concentration of DNA were determined by NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA). Subsequently, all samples were genotyped with the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA).

Genotype quality control was implemented using the SNPStats package in R software (Solé et al., 2006). Samples with a call rate <0.90 and heterozygosity of 3.0 standard deviations above or below than the observed mean were removed. For the quality control of mapped SNP, only autosomal SNP with call rate >0.90 and minor allele frequency >0.03 were considered. After this quality control, the genotype file contained 40,283 SNP markers and 476 samples, including 4 Holstein bulls, 23 Gir cows, 65 F₁, and 384 F₂ animals. Missing genotypes were imputed using FImpute software (Version 2.2) to ensure better statistical analysis effectiveness (Sargolzaei et al., 2011).

Statistical Analyses

Because the tick count data did not follow normal distribution, they were normalized using a natural log-transformation: $\ln(\text{tick count} + 1)$. The bivariate model used was

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{W}\mathbf{a} + \mathbf{e},$$

where $\mathbf{y}$ is the observations vector of the transformed tick count in rainy and dry seasons; $\boldsymbol{\beta}$ is the vector of fixed effects (i.e., contemporary group, sex, coat color and hair type, and the covariates, such as age, coat thickness, length and hair density); $\mathbf{a}$ is the vector of random additive genetic effects, assuming $\mathbf{a} \sim N(0; \mathbf{G}_0)$

$\otimes \mathbf{H}$), where $\mathbf{H}$ is a matrix combining genomic and pedigree information, as proposed by Aguilar et al. (2010) and $\mathbf{G}_0$ is the covariance matrix related to the additive genetic effect; $\mathbf{X}$ is the incidence matrix of fixed effects; $\mathbf{W}$ is the incidence matrix of random additive genetic effects; and $\mathbf{e}$ is the incidence vector of residual effect, assuming $\mathbf{e} \sim N(0; \mathbf{R}_0 \otimes \mathbf{I})$, where $\mathbf{I}$ is the identity matrix and $\mathbf{R}_0$ is the covariance matrix related to the residual effect.

Analyses for estimation of variance components and genetic parameters, as well as the GWAS, were performed using the single-step BLUP procedure (Aguilar et al., 2010), as implemented in AIREMLF90 from the BLUPF90 family of programs (<http://nce.ads.uga.edu/>; Misztal et al., 2002). To map the loci explaining genetic differences in the evaluated trait linkage disequilibrium, blocks were identified in the genome based on linkage disequilibrium, genetic variance was estimated in 0.5-megabase Mbp overlapping windows using PostGSf90 software (<http://nce.ads.uga.edu/>; Aguilar et al., 2011). Percentage of additive genetic variance explained by window has been calculated based of genetic value of the each window of 0.5 Mbp and the total additive genetic variance, as described by Wang et al. (2014). According to Habier et al. (2011), windows capture the QTL effect better than a single SNP, as several SNP are in linkage disequilibrium with a QTL, where each of these SNP explains a part of the QTL effect, allowing more precise estimation of this effect in a given chromosomal region. For the Manhattan plots construction, we selected nonoverlapping windows that exhibit the highest genetic variance using the mhtplot function of R software (version 3.3.1) to illustrate the genetic variance explained by each window.

Gene Search and Gene Network Building

According to Wang et al. (2014), the top 10 windows based on genetic variance were selected for each evaluated season (dry and rainy) to identify candidate genes from the initial and final coordinates of the each selected windows. Gene database of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) was used to identify candidate genes located in the selected regions based on the UMD 3.1 assembly (Zimin et al., 2009) of the bovine genome.

From the identified candidate gene sets (rainy and dry season), we excluded noncoding RNA genes; those remaining were used for biological processes enrichment analyses. For that, the ClueGO application in Cytoscape (Bindea et al., 2009) was used based on a unilateral hypergeometric test and Bonferroni correction, which

simultaneously analyzes 1 or more sets of genes and searches for a functional Gene Ontology (GO) term or pathways that establish relationships among genes.

Besides from the gene sets, promoter sequences (FASTA format) were obtained by considering 3,000 bp upstream and 300 bp downstream from gene transcription start sites (Verardo et al., 2016) based on the bovine genome. These data were used as input to the TFM-explorer program (<http://bioinfo.lifl.fr/TFM/TFME/>) for the identification of enriched TF. This program uses weighting matrices from JASPAR database (<http://jaspar.binf.ku.dk/>; Sandelin et al., 2004) to detect all potential transcription factor binding sites (TFBS) of a set of gene sequences and search for overrepresented TFBS. This program extracts significant groups (TFBS regions of the selected gene sequences associated with a factor) by calculating a scoring function threshold that is chosen to give a *P*-value equal to or less than 10^{-3} for each position in each sequence, as described in Touzet and Varré (2007).

The TF list was then analyzed in Cytoscape software (Shannon et al., 2003) using the Biological Networks Gene Ontology tool (Maere et al., 2005) to determine significantly overrepresented functional GO terms. Based on the overrepresented biological processes in Biological Networks Gene Ontology tool and evidence of literature review related to the studied trait, we were able to identify the TF most related to tick resistance, allowing us to generate a gene-TF interaction network. Gene-TF network analysis was performed in Cytoscape software based on the number of TFBS (connections with the selected TF) in each gene to determine the strongest associations with tick counts that will aid in the identification of the most likely candidate genes.

Assignment of Breed of Origin of Alleles

To assign the genetic origin of marker alleles in the F_2 population, the breed origin of alleles (BOA) approach (Vandenplas et al., 2016) was used with the parameter settings recommended by Sevillano et al. (2016). The BOA approach consists of 3 steps, namely (1) phasing of pure and crossed animal haplotypes; (2) determining the unique haplotypes for each pure breed; and (3) assigning the breed of origin to alleles observed in F_2 animals based on the determined unique haplotypes.

The software AlphaPhase 1.1 (Hickey et al., 2011) was chosen for haplotype phasing. The phasing step was performed using pedigree, and during the second step of the BOA approach a relaxation factor of 20% was used, which represents the maximum percentage of haplotype copies that can be observed in a popula-

tion of a different breed. If the percentage of haplotype copies identified in a single breed is less than (100 – relaxation factor), the breed of origin of this haplotype is defined as unknown.

Crossing between *Bos taurus* and *Bos indicus* animals results in the formation of long chromosome segments in F₂ crossed animals, which are expected to have the same origin. These segments allow a clear classification of origin of the SNP as *Bos taurus* or *Bos indicus* (Bolormaa et al., 2013).

After the origin of alleles analysis for the entire population, F₂ animals were classified as resistant (10% of animals with the lowest genomic breeding values for the number of ticks) or susceptible (10% of animals with the highest genomic breeding values). In these selected samples, the adjacent SNP close to genes in the networks were used to identify the origin of their alleles. The SNP alleles from each group were assigned to their breed of origin and paternity and classified into 4 types: father H (Holstein), father G (Gir), mother H (Holstein), and mother G (Gir). After this classification, the alleles of each SNP were quantified and classified according to the breed of origin using the Chi-squared test to compare the segregation between resistant and susceptible groups.

RESULTS AND DISCUSSION

Heritability estimates for tick counts were 0.40 ± 0.11 in the rainy season and 0.54 ± 0.11 in the dry season. In other studies, heritability for tick resistance ranged from 0.09 to 0.37 (Prayaga et al., 2009; Machado et al., 2010; Turner et al., 2010). In a previous study using 180 microsatellite markers in this same F₂ experimental population, Machado et al. (2010) estimated a lower heritability (0.21 ± 0.12). A total of 40,283 SNP markers were used, which allowed identifying a greater number of QTL regions for tick count, contributing to the estimation of a higher heritability. The different statistical models applied in each experiment may also explain the different estimates of heritability in both studies. Moreover, Machado et al. (2010) analyzed the data using a single-trait model, so the correlation (0.36) between counts made in the same individual in 2 seasons was estimated using Pearson product-moment correlation coefficient. On the other hand, we estimated the genetic correlation (0.96) between tick count in each season using bivariate analyses. Independent of the statistical methodologies and molecular tools used to assess animal genotypes, our results indicate the presence of genetic variability for tick counts. According to Turner et al. (2010), the selection for resistance would not affect the milk production in cattle. Those authors

identified a low correlation between the effects of alleles involved in milk production and tick counts in an association study using SNP in *Bos taurus* animals from Australia. Prayaga et al. (2009) also reported low and nonsignificant genetic correlation between tick counts and several production traits, confirming that selection for this trait does not promote unfavorable effects on other economically important traits.

Based on GWAS results, we identified 7,327 windows with mean density of 9 SNP per window (Figures 1 and 2). We selected 10 windows with the highest percentage of genetic variance explained distributed in chromosomes 10 and 23. Other studies, which evaluated tick resistance in cattle, also reported several QTL on the same chromosomes (Machado et al., 2010; Turner et al., 2010; Mapholi et al., 2016). All windows identified in the rainy season were also found in the dry season, differing only by the genetic variance explained by each window (Table 1). The window with the highest genetic variance was located in *Bos taurus* autosome 23 and was the same for both seasons, explaining 1.80 and 1.84% for the rainy and dry seasons, respectively. In the previous study performed by Machado et al. (2010), the identified QTL showing the highest phenotypic variance was also found on chromosome 23 for both seasons. The other windows followed the same pattern of additive genetic variance between both seasons, a fact that might be due to the high genetic correlation (0.96) between the traits evaluated for dry and rainy seasons. The percentage of genetic variances explained by all selected windows is noticeable, 5.34% for each season, and suggests the existence of a strong relationship between these genome regions and the resistance to bovine tick in this population.

From the windows selected in both seasons, 60 genes encoding proteins with different functions were annotated. To better understand the functions of these tick resistance genes, we performed a biological process analyses (Figure 3). In this GO network, we identified genes biological process enrichment involved in relevant immune functions, such as *TREM1*, *TREM2* (window 6, Table 1), and *CD83* (window 10, Table 1). Triggering receptor expressed on myeloid cells (*TREM*) are important regulators of immune response, as they are potent amplifiers of inflammatory responses (Genua et al., 2014). Gene *TREM1* has been related to monocytes and neutrophils blood circulating, macrophages, dendritic cells (DC) and activation of T-cells, CD86, and major histocompatibility complex (MHC) class II (Schenk et al., 2007; Chen et al., 2008; Sharif and Knapp, 2008). The expression of *TREM2* was detected in DC, monocytes, and macrophages (Turnbull et al., 2006; Seno et al., 2009; Bosco et al., 2011); DC, along

with CC chemokine receptors 7, increase expression in dendritic cells of costimulatory molecule (B7) and MHC class I and II (Gao et al., 2017).

The *CD83* gene is an immunoglobulin superfamily member and is regulated during DC activation, being used to identify the maturation or activation of DC in peripheral circulation (Breloer and Fleischer, 2008). Gene *CD83* is expressed in most immune cells such as B cells (Kretschmer et al., 2007), monocytes and macrophages (Cao et al., 2005), neutrophils (Yamashiro et al., 2000), and a regulatory subset of natural killers cells (Mailliard et al., 2005), thus showing its essential role in the initiation and regulation of innate and adaptive immune responses. It acts in the stimulation of immature and memory T cells by activation of DC (Aerts-Toegaert et al., 2007), thymic maturation and activation of CD4⁺ and CD8⁺ (Ju et al., 2016), maturation and maintenance of peripheral lymphocyte B homeostasis (Lüthje et al., 2008), and in the increased expression of MHC class I and CD86 (Tze et al., 2011). Thus, this gene also has functions potentially involved with the physiological response to tick infestation in

cattle, what makes it a candidate gene for this trait along with *TREM1* and *TREM2*. Carvalho et al. (2014) identified potential tick resistance genes from this same family (*CD14*, *CD5L*, *CD55*, *CD68*, *CD84*, *CD86*) when analyzing gene expression microarrays in the first hours of tick infestation in the same experimental population.

We performed in silico analyses of regulatory sequences for all detected genes and found 25 significantly enriched TF ($P < 0.0001$). The TF list was analyzed to determine which functional GO terms were significantly overrepresented. The TF most associated with tick counts, based on biological processes and literature review (Table 2), were then chosen to generate a gene-TF network (Figure 4), which allowed the identification of candidate genes for tick resistance.

Among the analyzed TF in the network, 2 nodes were linked to a high number of genes (*TAL1::TCF3* and *PAX5*). The *TCF3* transcription factor is a regulator of lymphopoiesis B and acts in the first detectable stages of B-cell involvement, development, and maturation (van der Veer et al., 2014). It is also an important regulator of T cell development and its deficiency in

Figure 1. Manhattan plot of additive genetic variance explained by nonoverlapping windows in the experimental Holstein $\times$ Gir F₂ population evaluated for cattle resistance to tick in the rainy season. Color version available online.

Table 1. Top 10 selected windows for percentage of additive genetic variance for the rainy and dry seasons in an experimental Holstein × Gir F₂ population evaluated for cattle resistance to tick

Window	Chromosome	Start ¹	End ²	Var. Exp. Rainy ³	Var. Exp. Dry ⁴
1	23	30,806,829	31,286,064	1.80	1.84
2	23	9,712,645	10,167,082	0.68	0.69
3	23	42,206,374	42,669,248	0.46	0.44
4	10	57,588,822	58,054,825	0.40	0.41
5	23	12,997,137	13,484,531	0.39	0.38
6	10	54,341,869	54,787,499	0.34	0.33
7	10	33,343,859	33,774,666	0.34	0.36
8	23	35,156,681	35,642,738	0.32	0.32
9	23	19,915,536	20,402,236	0.30	0.29
10	23	14,992,700	15,465,413	0.30	0.30

¹Position in base pair of the first SNP in the window.²Position in base pair of the last SNP in the window.³Percentage of additive genetic variance explained by each window for rainy season.⁴Percentage of additive genetic variance explained by each window for dry season.

mice leads to defects in the selection and differentiation of thymocytes (Aspland et al., 2001). The *TAL1* is the master regulator of hematopoietic stem/progenitor cells in humans (Benyoucef et al., 2015) and it is essential to formation but not involved in the maintenance

of hematopoietic stem cells in adults (Capron et al., 2006). Gene *PAX5* is involved in the entry of common lymphoid cells into B cell line. In the absence of *TCF3*, *PAX5* is responsible for in vivo activation of B lymphocyte transcription program (Souroullas et al., 2009).

Figure 2. Manhattan plot of additive genetic variance explained by nonoverlapping windows in the experimental Holstein × Gir F₂ population evaluated for cattle resistance to tick in the dry season. Color version available online.

Figure 3. Functional networks showing gene interactions (triangle nodes) related to cattle tick resistance in dry and rainy seasons and the relationship across genes and their subnetworks related to immune system biological processes. Color version available online.

We also found 4 TF showing a high number of connections in the gene-TF network: *NFIL3*, *EGR1*, *SOX10*, and *REL*. Gene *NFIL3* is believed to be associated with the function of adaptive immune lines and natural killer cell development (Seillet et al., 2014). Genes *EGR1* (Carter et al., 2007), *SOX10* (Fufa et al., 2015), and

REL (Hayden and Ghosh, 2011; Herrington and Nibbs, 2016) were found to be associated with prereceptor-mediated differentiation of thymocytes, proliferation and survival of melanocytes and melanogenesis, and key regulation of inducible gene expression in the immune system, respectively.

Table 2. Enriched transcription factors (TF) associated with genes identified for cattle tick counts in rainy and dry season, based on their biological process and literature review (data obtained in an experimental Holstein × Gir F₂ population evaluated for bovine tick resistance)

TF ¹	Biological process	Literature review ²
TAL1::TCF3	Immune system processes	Lymphocyte B regulator (Aspland et al., 2001) and T cell development (van der Veer et al., 2014)
NFIL3	Immune system processes	Adaptive immune lines and natural killer cell development (Seillet et al., 2014)
EGR1	Immune system processes	Differentiation of thymocytes mediated by T cell prereceptors (TCR) (Carter et al., 2007)
SOX10	Pigment cell differentiation	Proliferation and survival of melanocytes and melanogenesis (Fufa et al., 2015)
REL	Regulation of IL-12 biosynthesis processes	Key regulation of inducible gene expression in the immune system (Hayden and Ghosh, 2011; Herrington and Nibbs, 2016)
PAX5	Immune system processes	Entry of common lymphoid progenitors (CLP) into the B cell line (Souroullas et al., 2009)
PBX1	Immune system processes	Transformation of pre-B cells (Aspland et al., 2001)

¹TAL1 = T-cell acute lymphocytic leukemia 1; TCF3 = transcription factor 3; NFIL3 = nuclear factor, interleukin 3 regulated; EGR1 = early growth response 1; SOX10 = SRY (sex determining region Y)-box 10; REL = REL proto-oncogene, NF-κB subunit; PAX5 = paired box 5; PBX1 = pre-B-cell leukemia transcription factor 1.

²The cited literature studies are just a sample from the vast available literature.

From the gene-TF network, candidate genes for tick counts were highlighted based on TFBS. The *MYO5A* gene (window 3, Table 1) showed connections to a set of TF (*TAL1::TCF3*, *NFIL3*, *EGR1*, and *PAX5*). This gene encodes the myosin Va protein found in melanocytes and, along with other proteins, assists in the melanosomes transport, which are structures that produce the melanin pigment responsible for pigmentation of skin, hair, and eyes (Pastural et al., 2000; Hume

and Seabra, 2011). Melanocytes play an important role in the innate immune response, controlling microbial infection and inflammation, as these cells contribute to phagocytosis of invading pathogens. As they present antigen to the competent immune cells, melanocytes produce key cytokines, such as IL-1 β , IL-6, and tumor necrosis factor- α , as well as chemokines, which alert macrophages, neutrophils, and T cells. Melanocytes can further express pattern recognition receptors, which

Figure 4. Gene-transcription factor (TF) network: genes located in the top 10 windows for tick counts in dry and rainy seasons (circular/green nodes) and their associated TF (octagonal/yellow nodes). The node size corresponds to the network analyses (Cytoscape; Shannon et al., 2003) in which the larger nodes correspond to a higher edge density associated with the number of TF binding sites. Square/blue nodes are the biological processes related to TF (Gene Ontology terms). Data obtained in an experimental Holstein $\times$ Gir F_2 population evaluated for cattle tick resistance. Color version available online.

detect specific pathogenic structures and induce the production of Type I IFN and other immune system proteins (Gasque and Jaffar-Bandjee, 2015).

The *TREML1* gene (window 6, Table 1) showed connection to *TAL1::TCF3*, *REL*, *PAX5*, and *EGR1* TF. The TREM-like transcript-1 (*TLT-1/TREML1*) belongs to the *TREM* family and is known to be selectively expressed in activated platelets and to facilitate platelet aggregation by binding to fibrinogen, acting as a chemotactic mediator in the attachment of thrombi to endothelium vascular (Esponda et al., 2015). Its soluble form (*sTLT-1*) participates in the regulation of inflammation during sepsis, attenuating leukocyte activity and modulating the interference between platelets and neutrophils (Morales et al., 2010; Derive et al., 2012).

The *PRSS16* gene (window 8, Table 1), which did not share any ontology in the biological processes network (Figure 3), was related to the most enriched TF (*TAL1::TCF3* and *PAX5*) and shows evidence of being associated with the immune system (Brisson et al., 2015). The importance of this gene is paramount in the positive regulation of CD4⁺ T lymphocytes, as it encodes the thymus-specific serine protein, which is predominantly expressed by the thymic epithelial cells in the cortex, and is involved in the generation of peptides presented by class II MHC molecules in the thymus (Viret et al., 2011b; Fornari et al., 2012). Thymus-specific serine protein is required for the intracellular development of cells expressing T cell receptors, contributing to the diversification of T cell receptors repertoire of endogenous functional CD4 T cells in the thymus (Viret et al., 2011a). These genes are also involved in the organism defense against pathogens and injuries, and thus may be involved in bovine resistance against ticks.

Among all genes of the immune system associated with the host resistance to pathogens, the major histocompatibility complex genes [MHC or bovine lymphocyte antigen (**BoLA**)] are the most studied. Located on chromosome 23, these genes encode cell surface glycoproteins, which work as receptors on antigen presenting cells, connecting and presenting antigenic peptides to T lymphocytes, thus triggering the onset of immune response in the host (Tizard, 2008). In the same position on chromosome 23, Machado et al. (2010) found highly significant QTL associated with tick counts using the same experimental population as the current work. The association between MHC class II alleles and tick resistance was also reported by Martinez et al. (2006), who found an association between a low number of ticks and gene-BoLA-DRB 3.2, alleles 18, 20, and 27, suggesting that BoLA-DRB 3.2 alleles could be used to aid in the selection of tick-resistant animals. Other studies also reported a significant relationship between MHC genes

and tick counts (Acosta-Rodríguez et al., 2005; Untalan et al., 2007). Most of the genes identified in our study (e.g., *TREM1*, *TREM2*, *CD83* and *PRSS16*) showed important roles associated with the activities of MHC class I and II, suggesting that these genes could be involved in the host immune response to tick infestation.

Based on the networks, we selected genes to assess the origin of alleles associated with bovine tick resistance. Considering the LD between SNP and genes, the physical position of *TREM1*, *TREM2*, *CD83*, *MYO5A*, *TREML1*, and *PRSS16* were annotated for adjacent SNP selection. We selected a total of 11 SNP: 3 SNP in window 3, 4 in window 6, 2 in window 8, and 2 in window 10 (Table 1). The animals were grouped as resistant or susceptible animals based on their genomic breeding values. Thus, the alleles of the selected SNP were classified and quantified according to their breed of origin and paternity: HH for both paternal and maternal alleles originated from Holstein breed, GG for both paternal and maternal alleles originated from Gir breed, HG for paternal allele originated from Holstein breed and maternal allele from Gir breed, and GH for paternal allele originated from Gir breed and maternal allele from Holstein breed. According to de Roos et al. (2009), the effects of SNP on the phenotype depend on the linkage disequilibrium between marker and QTL, but this linkage disequilibrium can differ between *Bos taurus* and *Bos indicus*, as they are distinct subspecies, which makes the search for the origin of alleles extremely important.

Based on Chi-squared test applied to resistant and susceptible animal genotypes, we observed 3 significant SNP ($P < 0.05$) in resistant animals (SNP 8, 10 and 11) and 9 in susceptible animals (SNP 1 to 9) (Table 3), which showed a deviation of the expected gametic segregation of 25% for each genotypic class (1 HH, 1 GG, 1 HG, 1 GH). In resistant animals, the average proportion of animals for GG significant SNP was 44.8%, whereas animals classified as HH were represented by only 11.3%. On the other hand, in susceptible animals, HH represented 44.6%, on average, whereas GG animals represented 9.3% of the animals in this group. Based on these findings, it was observed that most resistant animals inherited both Gir alleles, whereas a small part received both Holstein alleles. Machado et al. (2010), evaluating the same experimental population as our study, identified a QTL with a negative additive effect on chromosome 5 from Holstein breed, showing that alleles from this susceptible breed also contributed to the increased resistance of the evaluated animals for tick resistance.

In the 2 evaluated groups, animals classified as HG and GH represented 43.9 and 46% of resistant and susceptible animals, respectively. As we were not able to

Table 3. Percentage of individuals classified by breed of origin and paternity of each SNP allele analyzed in tick resistant and susceptible animals in an experimental Holstein × Gir F₂ population

Alleles	Window 3			Window 6				Window 8		Window 10	
	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11
Resistant animals											
HH ¹	11.8	12.1	12.5	18.2	18.2	20.0	20.7	16.7*	17.1	8.6*	8.8*
GG ²	38.2	39.4	37.5	39.4	39.4	33.3	34.5	44.4*	42.9	45.7*	44.1*
HG ³	29.4	27.3	28.1	27.3	27.3	30.0	27.6	25.0*	25.7	31.4*	32.4*
GH ⁴	20.6	21.2	21.9	15.2	15.2	16.7	17.2	13.9*	14.3	14.3*	14.7*
Susceptible animals											
HH	42.1*	40.5*	42.1*	41.2*	41.9*	41.9*	43.8*	54.1*	54.1*	43.3	45.2
GG	10.5*	10.8*	10.5*	5.9*	6.5*	6.5*	6.3*	13.5*	13.5*	23.3	22.6
HG	31.6*	32.4*	31.6*	32.4*	29.0*	29.0*	28.1*	16.2*	16.2*	13.3	12.9
GH	15.8*	16.2*	15.8*	20.6*	22.6*	22.6*	21.9*	16.2*	16.2*	20.0	19.4

¹Paternal and maternal alleles originated from Holstein breed.

²Paternal and maternal alleles originated from Gir breed.

³Paternal allele originated from Holstein breed and maternal from Gir breed.

⁴Paternal allele originated from Gir breed and maternal from Holstein breed.

*Significant SNP for allele origin in the chi-squared test ($P < 0.05$).

identify influence of the origin and paternity breed of the alleles in the SNP evaluated in these 2 classes, the phenotypes could be influenced by other factors not detected by our study, such as the presence of regulatory genes. As tick resistance is a polygenic trait, other genes with lower and epistatic effects, not detected in this GWAS analysis, may also have influenced this phenotype.

In summary, GWAS allowed us to identify candidate genes for tick resistance in an experimental F₂ (Gir × Holstein) population. The network analysis highlighted gene interactions that were consistent with the biology of the immune system and provided known TF that allowed the identification of candidate genes for cattle tick resistance in both rainy and dry seasons (e.g., *TREM1*, *TREM2*, *TREML1*, *CD83*, *MYO5A*, and *PRSS16*). According to origin of allele analysis, we observed that most of the animals classified as resistant showed 2 alleles from Gir breed, whereas the susceptible animals showed alleles from Holstein breed. Based on these results, the identified genes may be deeply investigated in additional experiments aiming to validate their effects on tick resistance phenotype in cattle.

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