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Genome-wide scan reveals important additive and non-additive genetic effects associated with resistance to *Haemonchus contortus* in Florida Native sheep

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ABSTRACT

Florida Native sheep is among the sheep breeds best adapted to humid and hot climatic conditions such as those of Florida, USA, and have shown a superior ability to regulate nematode burdens. This is one of the oldest sheep breeds in North America and is an endangered species. To ensure genetic diversity and long-term survival of the breed, protection of the current genetic stock is critical and conservation efforts are required to promote its breeding and production. The objective of the present study was to investigate the importance of additive and non-additive genetic effects on resistance to natural *Haemonchus contortus* infections in Florida Native sheep using a whole genome scan. A total of 200 sheep were evaluated in the present study. Phenotypic records included faecal egg count (FEC, eggs/gram), FAMACHA® score, packed cell volume (PCV, %), body condition score and average daily gain (ADG, kg). Sheep were genotyped using the GGP Ovine 50K SNP chip and 45.2 k single nucleotide polymorphism (SNP) markers spanning the entire genome were available for quality control procedures. Mixed models were used to analyse the response variables and included the identity by state matrix to control for population structure. Bonferroni correction was used to control for multiple testing and a second arbitrary threshold (0.1×10^{-3}) was used. Fifteen SNPs with additive and non-additive genetic effects and located in *Ovis aries* chromosome OAR1, 2, 3, 6, 8, 10, 11, 12, 13 and 21 were associated with FEC, FAMACHA® score, PCV and ADG. These SNPs could be potential genetic markers for resistance to natural *H. contortus* exposure in Florida Native sheep.

1. Introduction

In the southern US and worldwide, the major limiting factor affecting grazing sheep systems is gastrointestinal nematode (GIN) infections which reduce performance and cause weight loss and anaemia (Miller et al., 1998). Economic losses due to GIN have a great impact on Florida Cracker sheep production systems, especially for lambs and susceptible adult sheep (Fig. 1). As observed in Fig. 1, sheep susceptible to GIN infections are not suitable for human consumption and are usually retained by the U.S. Department of Agriculture (USDA) meat inspectors, which impacts the economy of sheep producers.

Infection with gastrointestinal nematodes involves dynamic events where the immune response undergoes extensive CD4+T cell activation and cytokine production (Venturina et al., 2013). In sheep, the onset of GIN triggers the expression of genes and pathways associated with complement activation and Th1/Th2 immune responses to promote nematode rejection and damage of the nematode's cuticle (MacKinnon et al., 2009; Venturina et al., 2013; McRae et al., 2015; Estrada-Reyes et al., 2017, 2018). Host genetic variation allows selection of sheep with parasite resistance. Selection for parasite resistance offers several benefits to long-term sustainable sheep production and reduces anthelmintic treatment (Bishop, 2012). These benefits include permanent genetic change, improvement of animal performance and reduced infectivity of pastures. Thus, selective breeding for parasite resistance is the most sustainable strategy for improving sheep production in the southern US and worldwide.

Several sheep breeds identified as resistant to GIN in the southern US are indigenous breeds that have been developed under natural selection for many centuries with little or no anthelmintic

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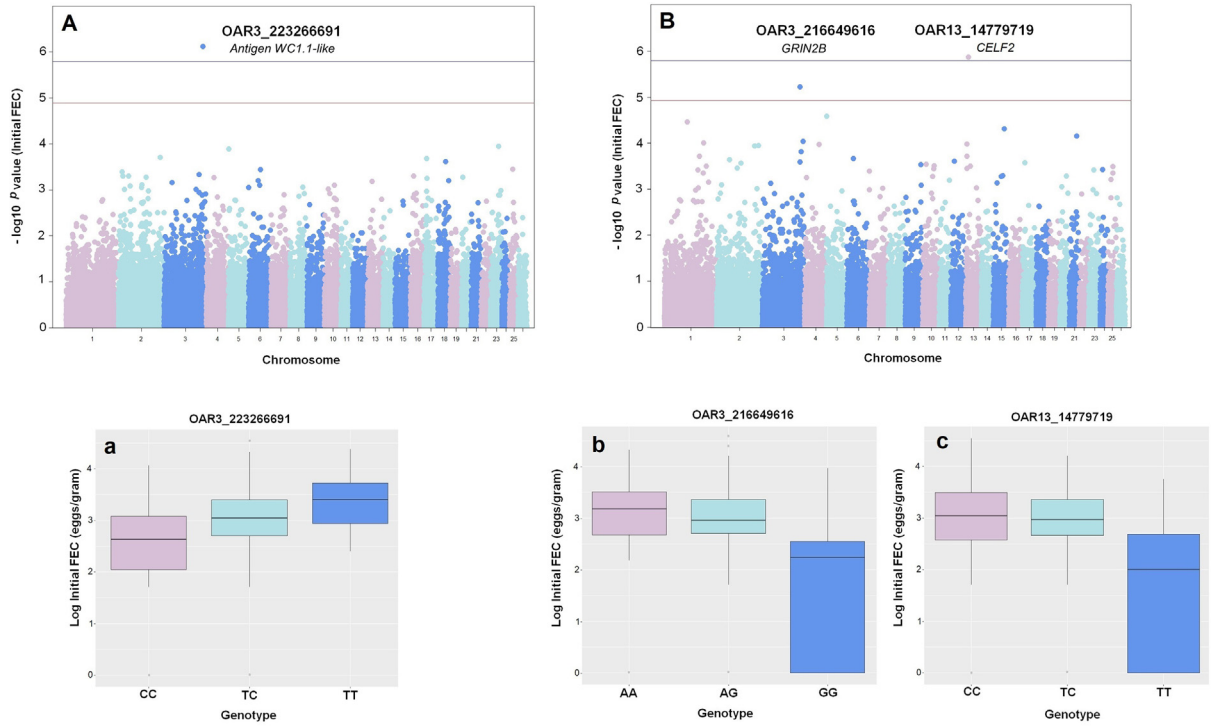

Fig. 1. Additive (A) and non-additive (B) genetic effects for initial faecal egg counts (FEC) in Florida Native sheep in Florida, USA, naturally infected with *Haemonchus contortus*. Box plots show the distribution of the significant single nucleotide polymorphisms (SNPs) with additive (a) and non-additive (b and c) genetic effects.

treatment. These breeds include Florida Native and Gulf Coast Native sheep (Loggins et al., 1965; Bradley et al., 1973; Zajac et al., 1988; Amarante et al., 1999a, 1999b; Miller et al., 1998; Li et al., 2001). Florida Native sheep (also known as Florida Cracker sheep) is one of the oldest sheep breeds in North America. This breed is the most adapted to the humid and hot climatic conditions of Florida and express superior ability to regulate nematode burdens (Amarante et al., 1999a, 1999b; Estrada-Reyes et al., 2019a). However, sheep numbers are in decline and this breed is currently included in the endangered list of sheep breeds (http://livestock-conservancy.org/), thus, conservation efforts are required to protect the current genetic stock and promote their breeding and production.

It is believed that this heritage breed was developed from Churra sheep imported by the Spanish during the discovery of Florida and the foundation of St. Augustine from 1521 to 1565. The sheep that stayed in Florida became feral and their phenotypic attributes were re-shaped by natural selection for more than 400 years and then, when Florida ended the open range at the end of the Second World War, sheep were re-domesticated (https://livestockconservancy.org/index.php/heritage/internal/cracker-sheep). The re-domestication process included selective breeding for parasite resistance by producers from Florida using a faecal egg count (FEC) or a FAMACHA® score. Some of the biological mechanisms associated with parasite resistance in this breed include immune response events related to innate and acquired immune response (Estrada-Reyes et al., 2019a). However, the genetic architecture controlling resistance to GIN is still unknown.

Evidence from previous studies using Dorper, Katahdin and St. Croix sheep suggests that selective breeding for parasite resistance occurs under directional selection (Estrada-Reyes et al., 2019b) and this process has been widely used to increase the frequency of favourable additive alleles (Rieseberg et al., 2002). Several single nucleotide polymorphism (SNP)-based association studies have characterised novel genetic markers associated with parasite resistance in different sheep breeds (Kemper et al., 2011; McRae et al., 2014; Benavides et al., 2016; Sweeney et al., 2016). These genome-wide association studies (GWAS) have focused on additive genetic effects while non-additive effects have not been studied. It is possible that complex traits such as parasite resistance may involve several biological events that cannot be explained by simple additive models.

Candidate gene investigation of preselected genes related to the immune response against *H. contortus* was the first approach used to search for genetic markers associated with resistance to GIN in Florida Native sheep (Estrada-Reyes et al., 2019a). Results from this study revealed SNPs with additive genetic effects associated with the FEC, FAMACHA® score and haematological parameters. However, it is possible that other significant markers with greater additive and non-additive genetic effects across the sheep genome could be associated with resistance to GIN. Thus, the objective of this study was to investigate the importance of additive and non-additive genetic effects on resistance to natural *H. contortus* infections in Florida Native sheep using a whole genome scan.

2. Materials and methods

2.1. Population and phenotypic data

The research protocol was approved by the University of Florida Institutional Animal Care and Use Committee, USA (Approval number 201810108). The study was carried out in two consecutive years (2018–2019) during the summer season. The history of the Florida Native sheep population segregating for parasite resistance and susceptibility, and used in this study, has been described previously (Estrada-Reyes et al., 2019a). Briefly, animals used in the study were from a commercial Florida Native sheep farm located in Ocala, Florida, USA. Individuals have been selected for parasite resistance since 1998 on the basis of FEC phenotyping, FAMACHA® score and parentage recording. A total of 200 Florida Native sheep

were tested for GINs under natural grazing conditions. Animals born in 2018 and 2019 were grouped based on age (3–5 months old), and naturally exposed to parasites since birth. At the beginning of the study each year, initial FEC, FAMACHA score, weight, packed cell volume (PCV) and body condition score (BCS) were measured, and animals were dewormed with levamisole (18 mg per kg of body weight) the same day of initial screening. Then, animals returned to grazing conditions and 10 days post deworming, FEC reduction was confirmed and FAMACHA score, weight, and BCS were recorded. This date was used as the baseline (0 days) for future phenotype collection dates. Finally, 28 days post baseline (28 days), the same phenotypic variables were measured and average daily gain (ADG, kg) was determined based on weight and days under study.

Identification of gastrointestinal parasite eggs was carried out using fresh faecal samples collected directly from the rectum of evaluated sheep and McMaster's FECs were performed as described by Zajac and Conboy (2012) at start of the trial (initial FEC), during baseline (0 days) and 28 days post baseline (28 days). However, due to relatively constant numbers for the other species, only eggs of *Haemonchus* spp. were recorded and analysed.

The Shapiro–Wilk test was used to test phenotypic variables for normality. Box-Cox transformation was performed to obtain a normal distribution of values. The R "car" library was used to estimate the power parameter λ and carry out logarithmic transformation for FEC ($\log(x + 1)$). For all the traits, initial screening (day −10) and 28 days were included in the analysis.

2.2. Genotyping

Genomic DNA from blood collected from the jugular vein using vacutainer tubes with anticoagulant EDTA at baseline was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according the manufacturer's instructions and stored at −20 °C. The DNA yield was calculated from a spectrophotometric measurement at 260 nm (NanoDrop-1000, Thermo Scientific, USA), and the purity was assessed using a ratio 260/280 nm. All animals were genotyped with the commercial GGP Ovine 50K SNP chip (GeneSeek, Inc., Lincoln, NE, USA). A total of 45,205 SNPs were available for quality control procedures. Markers were removed based on genotype call rates per marker (<95%) and minor allele frequency (MAF) ≤ 0.05. Linkage Disequilibrium (LD) pruning was performed using the default options in the SNP & Variation Suite (SVS, Golden Helix, Inc., Bozeman, MT, USA). A total of 32,500 SNPs were available for analysis after quality control.

The array details and SNP marker statistics are available in Supplementary Data S1 and S2, respectively. The SNP information and genotypes from GGP Ovine 50K SNP arrays are presented in Supplementary Data S3. The total number of SNPs within each chromosome are presented in Supplementary Data S4.

2.3. Modelling additive and non-additive SNP effects

Considering a single SNP locus with two alleles (A and B), alternative genetic effects, namely additive, dominance and recessive, were tested. Additive effects were modelled using SNP genotypes coded as 0 for AA, 1 for AB and 2 for BB. For dominance effect estimation, SNPs were coded as 0 for AA, 1 for AB and BB. For recessive genetic effects, SNPs were coded as 0 for both AA and AB, and 1 for BB.

2.4. SNP-genome wide association study (GWAS)

The analysis was performed using the SNP & Variation Suite (SVS, Golden Helix, Inc., Bozeman, MT, USA). Mixed models to evaluate FEC (Initial and 28 days), FAMACHA® score (Initial and 28 days), PCV (Initial and 28 days) and ADG were implemented using the Efficient Mixed Model Association eXpedited (EMMAX) procedure (Kang et al., 2010). Fixed effects included SNP and a contemporary group conformed by age of the animal, sex, group and year. To control for population structure, the genomic relationship matrix was calculated from SNP information and included in the analysis as a random effect. The mixed model used for the association analysis was as follows:

$$y = xb + zu + Wa + e$$

where: y represents the vector of the phenotypic observation for each animal; b is the vector of fixed effects, u is the vector of random animal polygenic effect, $u \sim N(0, G\sigma^2_u)$, where G is the additive genomic relationship matrix; a represents the fixed SNP additive effect, and e represents the residual vector, $e \sim N(0, I\sigma^2_e)$. The matrices X, Z and W represent the incidence matrices for b, u and a, respectively. SNP markers were tested one at a time, and Bonferroni correction was used to control for multiple testing. If no SNP reached the genome wide threshold for a certain trait, a secondary arbitrary threshold of 0.1×10^{-3} was used.

2.5. Gene content and functional annotation

Genes with significant SNPs associated with the traits evaluated in this study were assessed using *Ovis aries* v1.0 and v4 in the Genome Data Viewer genome browser (https://www.ncbi.nlm.nih.gov/genome/?term=ovis+aries). The Quantitative Trait Locus (QTL) Database (https://www.animalgenome.org/cgi-bin/QTLdb/OA/index) was used to identify QTL regions (QTLs) that overlapped or were closer (<5 Mb) to significant SNPs. We investigated gene function using Gene Ontology (GO).

3. Results

3.1. Phenotypic data

The gastrointestinal parasite eggs identified were the following: *Haemonchus* spp, *Moniezia* spp, *Coccidia* spp, and *Strongyloides* spp. Table 1 shows the descriptive statistics for the traits available for this study.

3.2. Significant additive and non-additive effects

A total of 15 SNPs with additive or non-additive effects were significantly associated with FEC, FAMACHA® score, PCV and ADG (Table 2). The pattern of LD between these markers is shown in Supplementary Fig. S1.

Additive and non-additive effects observed for Initial FEC are presented in Fig. 1. For Initial FEC, a SNP (OAR3_223266691) located in the antigen WC1.1-like gene (LOC106991008) showed significant additive effects. Significant recessive effects were observed for two SNPs (OAR13_14779719 and OAR3_216649616) located in chromosomes 3 and 13, respectively. The OAR13_14779719 was identified in intron 2 of *CELF2* (CUGBP Elav-like family member 2) gene and the OAR3_216649616 was located close to *GRIN2B* (Glutamate ionotropic receptor NMDA type subunit 2B) gene.

For FEC 28 days, six SNPs (OAR1_140360039, OAR2_69370595.1 OAR3_217293778, OAR10_67638743, OAR11_20785327 and s05900.1) showed significant recessive effects (Fig. 2). These SNPs were identified in intron 3 of nuclear receptor interacting protein 1 (*NRIP1*), intron 1 of transient receptor potential cation channel subfamily M member 3 (*TRPM3*), Dead Box helicase 47 (DDX47), intron 12 of glypican 5 (*GPC5*), intron 5 of signal transducer and activator of transcription 5B (*STAT5B*) and uncharacterized LOC105603995

Table 1
Descriptive statistics for faecal egg count (FEC, eggs/gram), FAMACHA® score, packed cell volume (PCV, %), body condition score (BCS) and average daily gain (ADG) in Florida Native sheep in Florida, USA, naturally infected with *Haemonchus contortus*.

Trait	Time	*n*	Mean	S.D.	Min	Max
FEC (eggs/gram)	Initial	200	2322	441.1	0	35,050
	28 days	200	1797	323.9	0	19,300
FAMACHA® (score)	Initial	200	3	0.83	2	5
	28 days	200	3	0.80	1	5
PCV (%)	Initial	200	28	5.28	10	37
	28 days	200	29	4.40	15	37
BCS (score)	Initial	200	2.25	0.30	1.5	3
	28 days	200	2.27	0.10	1	3
ADG (kg)		200	0.16	0.07	0.05	0.35

Table 2
Significant single nucleotide polymorphisms (SNPs) associated with *Haemonchus contortus* faecal egg count (FEC, eggs/gram), FAMACHA score, packed cell volume (PCV, %) and average daily gain (ADG, kg) in Florida Native sheep in Florida, USA, naturally infected with *Haemonchus contortus*.

Trait	Marker	OAR	Genetic effects	Gene name	Position	SNP	−log10 p-value	β + SE	Proportion of variance explained	MAF	QTL ID
FEC (Initial)	OAR3_223266691	3	Additive	*LOC106991008 (WC1.1 like)*	223,266,691	C/T	6.04	2.54 ± 0.92	0.13	0.37	12939: *H. contortus* FEC
	OAR3_216649616	3	Recessive	*GRIN2β**	216,649,616	A/G	5.22	3.09 ± 0.92	0.12	0.43	12940: *H. contortus FEC**
	OAR13_14779719	13	Recessive	*CELF2*	14,779,719	C/T	5.89	2.99 ± 0.91	0.14	0.37	17198: *Salmonella abortusovis* susceptibility
FEC (28 days)	OAR1_140360039	1	Recessive	*NRIP1*	140,360,039	T/G	6.15	2.66 ± 0.97	0.14	0.36	95610: Faecal egg count
	OAR2_69370595.1	2	Recessive	*TRPM3*	64,886,088	A/G	6.07	2.60 ± 0.95	0.14	0.23	12898: Trichostrongylus adult and larva count
	OAR3_217293778	3	Recessive	*DDX47**	217,293,778	C/T	6.09	2.49 ± 0.96	0.15	0.42	12940: *H. contortus FEC**
	OAR10_67638743	10	Recessive	*GPC5*	67,638,743	C/T	5.72	2.63 ± 0.98	0.13	0.27	13989: Faecal egg count
	OAR11_20785327	11	Recessive	*STAT5β*	20,785,327	G/A	5.27	2.55 ± 0.98	0.14	0.15	12901: Trichostrongylus adult and larva count
	s05900.1	21	Recessive	*LOC105603995*	15,828,954	G/A	5.34	2.16 ± 0.97	0.13	0.43	95638: Immunoglobulin A level*
FAMACHA® (Initial)	OAR6_22599668.1	6	Dominance	*ARHGEF38*	19,651,819	G/A	5.14	2.55 ± 0.88	0.12	0.20	
	OAR8_23672340.1	8	Dominance	Unknown	23,672,340	G/A	5.40	2.31 ± 0.88	0.13	0.47	12899, 12900: Trichostrongylus adult and larva count
PCV (Initial)	s32184.1	2	Additive	*STC1**	44,214,401	G/A	5.62	29.89 ± 5.25	0.13	0.29	
PCV (28 days)	OAR12_25382580	12	Recessive	Unknown	25,382,580	G/A	5.05	30.01 ± 0.81	0.12	0.37	
ADG	OAR6_81571989	6	Recessive	*LOC101120245**	81,571,989	C/T	5.68	0.17 ± 0.03	0.14	0.30	14284: Body weight (Slaughter)

OAR, *Ovis aries* chromosome; MAF, minor allele frequency; [QTL, quantitative trait locus.

genes. The OAR11_20785327 was located downstream of a marker in the *STAT3* gene previously associated with FEC (Estrada-Reyes et al., 2019a).

For Initial PCV, significant additive genetic effects were observed for a SNP (s32184.1) located close to the stanniocalcin 1 (*STC1*) gene. Significant recessive effects were observed for a SNP (OAR12_25382580) associated with PCV 28 days (Fig. 3). This SNP was located downstream of the *TGFB2* gene.

Two SNPs (OAR6_22599668.1 and OAR8_23672340.1) with dominance effects were significantly associated with Initial FAMACHA® score (Fig. 4A). The SNP (OAR6_22599668.1) in intron 3 of the Rho guanine nucleotide exchange factor 38 (*ARHGEF38*) gene was located downstream of a marker in the complement factor I (*CFI*) gene previously associated with FEC (Estrada-Reyes et al., 2019a). One SNP (OAR6_81571989) located close to LOC101120245 (Receptor of activated protein C kinase 1 pseudogene) showed significant recessive effects associated with ADG (Fig. 4B).

3.3. SNPs identified in QTLs and GO information

Out of 14 SNPs, seven polymorphisms significantly associated with FEC, FAMACHA® score and ADG overlapped health and growth QTLs. The OAR1_140360039, OAR2_69370595.1, OAR3_223266691, OAR8_23672340.1, OAR10_67638743 and OAR11_20785327 that were significantly associated with FEC and FAMACHA® score, overlapped health QTLs related to *H. contortus* FEC and *Trichostrongylus* adult and larva counts (Table 2). Similar health QTLs were identified close to OAR3_216649616, OAR3_217293778 and s05900.1. The significant SNP

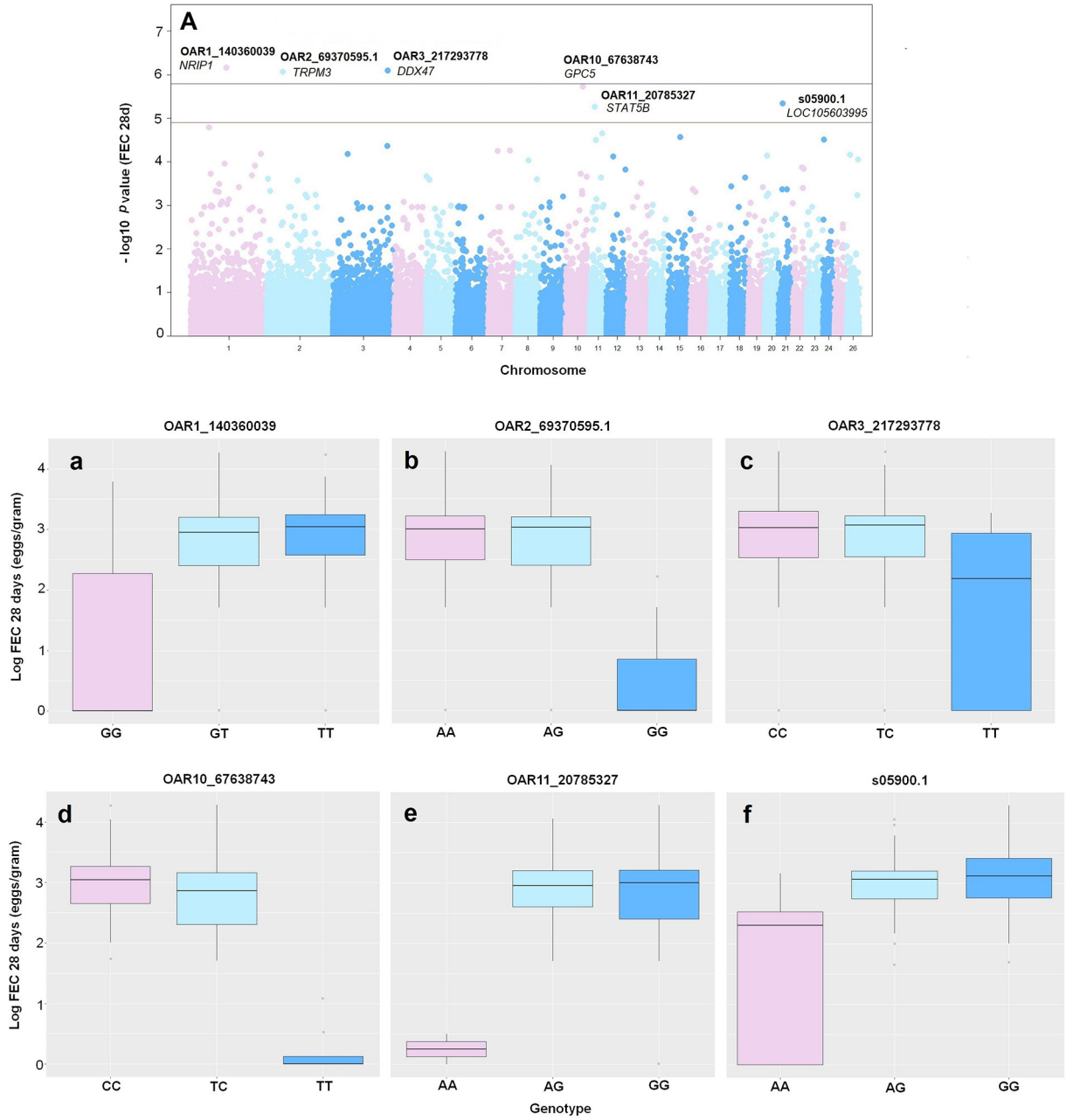

Fig. 2. Non-additive (A) genetic effects for faecal egg count (FEC) 28 days post baseline in Florida Native sheep in Florida, USA, naturally infected with *Haemonchus contortus*. Box plots show the distribution of the significant single nucleotide polymorphisms (SNPs) with non-additive (a, b, c, d, e and f) genetic effects.

(OAR6_81571989) associated with ADG overlapped a growth QTL related to body weight at slaughter and a health QTL related to FEC (Table 2).

GO analyses showed that some functions of the proteins encoded by these genes included biological events related to immune response mechanism, the Wnt signalling pathway and other cellular processes (Table 3).

4. Discussion

GIN infection is an important factor affecting sheep performance and health status in Florida. Improving resistance to GINs is one of the major goals in the sheep industry from the Southern US (and worldwide). Recent studies have identified and evaluated potential biomarkers for resistance to GINs in Florida Native sheep that could be used for selective breeding (Estrada-Reyes et al., 2019a). These studies have focused on genetic variants with additive effects, whereas the role of non-additive effects have not been elucidated. In dairy cattle, non-additive effects have been associated with sire conception rate and fitness related traits such as fertility (Nicolini et al., 2018). In sheep and other livestock species, it is possible that non-additive effects could be important for other complex traits such as parasite resistance. Results from our study showed that non-additive effects (recessive and dominant effects) could also play important roles in parasite resistance in Florida Native sheep. The majority of the SNPs associated with the traits evaluated (FEC, FAMACHA® score, PCV and ADG) presented non-additive effects and only two SNPs with additive effects were associated with Initial FEC and PCV, respectively.

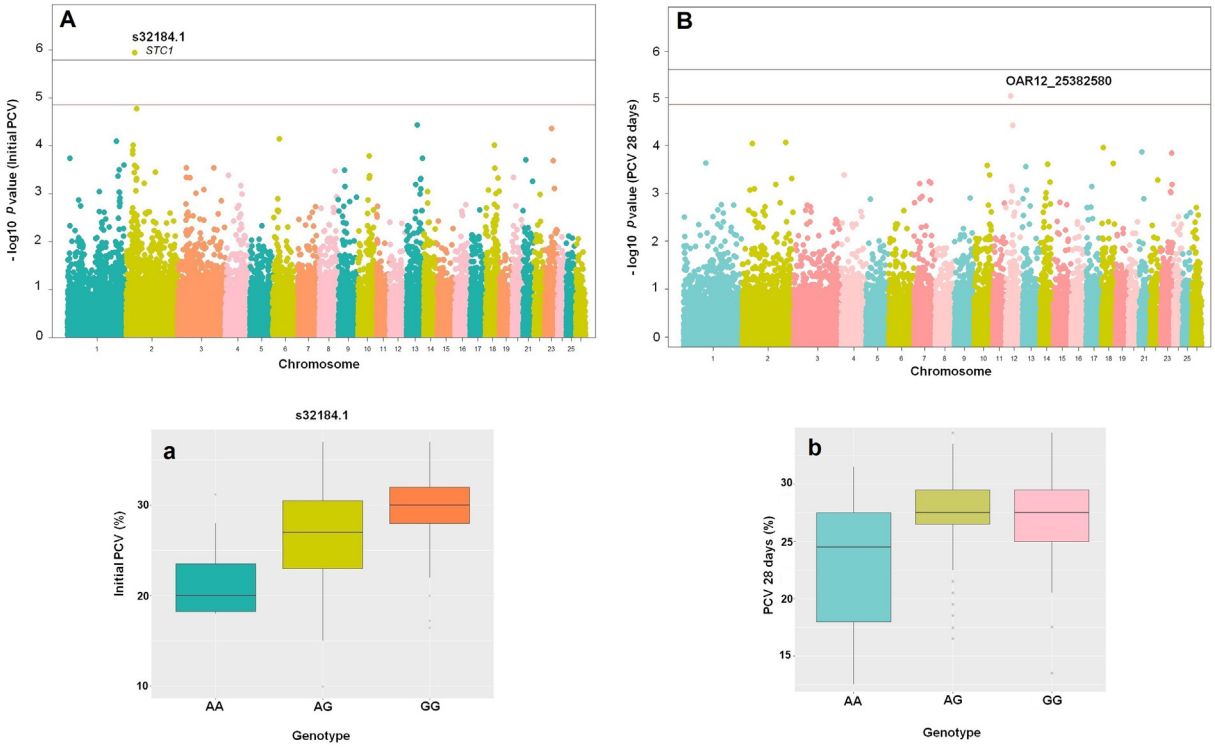

Fig. 3. Additive (A) and non-additive (B) genetic effects for packed cell volume (PCV) in Florida Native sheep in Florida, USA, naturally infected with *Haemonchus contortus*. Box plots show the distribution of the significant single nucleotide polymorphisms (SNPs) with additive (a) and non-additive (b) genetic effects.

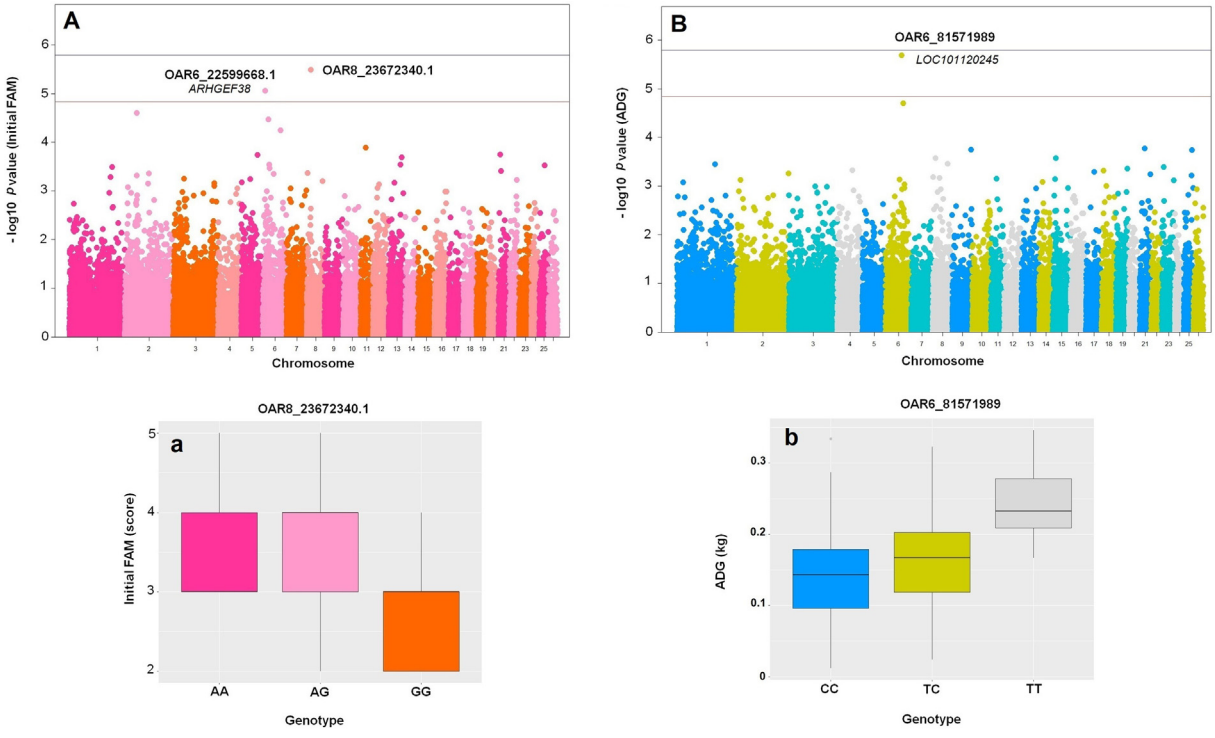

Fig. 4. Non-additive genetic effects for the FAMACHA® score (A) and average daily gain (ADG, B) in Florida Native sheep in Florida, USA, naturally infected with *Haemonchus contortus*. Box plots show the distribution of the significant single nucleotide polymorphisms (SNPs) with additive (a) and non-additive (b) genetic effects.

Genetic variations within antigen WC1.1-like, *CELF2, NR1P1, TRPM3* and *STAT5B* genes were associated with FEC. Antigen *WC1.1* gene encodes a receptor that is uniquely expressed on γδ-T cells and previous studies with resistant Canaria Hair sheep infected with *H. contortus* have suggested that the immune response modulated by WC1 + γδ- T cells (Supplementary Fig. S2) is the primary mechanism used for regulation of parasite growth and fecundity (González et al., 2011; Guo et al., 2016;

Table 3
The Gene Ontology (GO) terms for the genes with significant SNPs [single nucleotide polymorphisms] associated with *Haemonchus contortus* faecal egg count (FEC, eggs/gram), FAMACHA score, packed cell volume (PCV, %) and average daily gain (ADG, kg) in Florida Native sheep in Florida, USA, naturally infected with *Haemonchus contortus*.

Gene	GO term	Function
GRIN2β	GO:0035235	Ionotropic glutamate receptor signalling pathway
STAT5β	GO:0045588	Positive regulation of gamma-delta T cell differentiation
Antigen WC1	GO:0002682	Regulation of immune system process
TRPM3	GO:0016048	Transient receptor potential cation channel, subfamily M, member 3
NRIP1	GO:0001543	Ovarian follicle rupture
ARHGEF38	GO:0035025	Positive regulation of Rho protein signal transduction
GPC5	GO:0090263	Positive regulation of canonical Wnt signalling pathway

Hernández et al., 2017). CUGBP Elav-like family member 2, or the *CELF2,* gene encodes a RNA-binding protein that regulates post-transcriptional events (Han and Cooper, 2005) and the nuclear receptor interacting protein 1, or *NRP1,* gene encodes a coactivator of nuclear factor-κβ (NF-κβ) which is a master regulator of monocytes and macrophages (Ho et al., 2012). However, the roles of these genes during natural *H. contortus* infections in sheep are unknown. The *TRPM3* gene encodes a protein that mediates calcium entry and an increase in intracellular Ca^{2+} triggers activation of inflammatory pathways (Supplementary Fig. S3).

The *STAT5B* gene encodes a protein that mediates signal transduction triggered by cell ligands such as IL2, IL4, CSF1, and several growth hormones to promote diverse biological processes that include T-cell receptor (TCR) signalling (Taylor et al., 2006; Paul and Zhu, 2010) (Supplementary Fig. S4). Previous studies have observed that this gene is under directional selection and it is associated with resistance to *H. contortus* in sheep populations (Estrada-Reyes et al., 2019b). In goats, polymorphisms within *STAT5B* have been significantly associated with FEC (Alam et al., 2019).

For the FAMACHA® score, SNPs with dominance effects were significantly associated with this trait. One of these genetic variants was located downstream of the *CFI* gene, which contains a significant marker previously associated with FECs (Estrada-Reyes et al., 2019a).

For PCV, the SNP with additive genetic effects was located close to the *STC1* gene whereas the SNP with recessive genetic effects was located downstream of the *TGFB2* gene. STC1 controls calcium transport in intestinal epithelia and negatively regulates TRP channels (Xiang et al., 2016). The tumour growth factor beta 2 (*TGFB2)* gene encodes an anti-inflammatory cytokine highly concentrated in the gut mucosa of sheep infected with *H. contortus* (Robinson et al., 2011). Previous studies have observed that the *TGFB2* gene is essential for immune protection against *H. contortus* and it is under directional selection in sheep and goat populations (Estrada-Reyes et al., 2019b).

The SNP with recessive genetic effects and associated with ADG in our sheep population is close to the receptor of activated protein C kinase 1 pseudogene (RACK-1). The link between RACK-1 and ADG during *H. contortus* infections is unknown, however RACK-1 plays an important role in transporting and anchoring proteins at particular locations in the cell. It also interacts with the insulin-like growth factor I (IGF-I) receptor (IGF-IR) and regulates IGF-I-dependent integrin signalling (Hermanto et al., 2002). In lambs, plasma concentrations of IGF-I are related to growth and production (Roberts et al., 1990).

Overall, the significant SNPs associated with FEC, FAMACHA® score, PCV and ADG are related to important biological events that include: γδ- T cell signalling, cellular calcium signalling, NF-κβ activation and inflammatory response, immunity mediated by Treg and Th2 signalling, and growth. The majority of these markers overlapped previous QTLs associated with FECs. A wide range of markers associated with parasite resistance has been discovered using GWAS (Benavides et al., 2016), however, these studies suggest that a single panel of markers is not valid for all sheep breeds. It is possible that the mechanisms used for controlling *H. contortus* infection vary between sheep breeds but further studies would be required to evaluate this assumption. Our results provide additional evidence for the importance of additive and non-additive genetic effects in parasite resistance and suggest that FEC, FAMACHA® score, PCV and ADG are polygenic traits and not influenced by genes with major effects.

Despite the success of GWAS to identify genetic loci associated with parasite resistance in sheep (Kemper et al., 2011; McRae et al., 2014; Benavides et al., 2016; Sweeney et al., 2016; Estrada-Reyes et al., 2019a), the causal variants have been difficult to locate. It is possible that some of the significant SNPs or loci providing the strongest association signals may be tagging other co-inherited variants. For this reason, further validation of GWAS results is necessary to confirm the potential genetic markers controlling complex traits such as parasite resistance. After validation, functionality of these genetic variants could be studied through genome-editing techniques and utilising zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs), and clustered regularly-interspaced short palindromic repeats with Cas9 nuclease (CRISPR-Cas9) due to the ability to assess effects of single variants in vivo (Tait-Burkard et al., 2018). Some of these genome editing tools have been utilised in several livestock species (sheep, goat, pig, cattle and chicken) to target loci identified from GWAS and to improve muscle growth (Myostatin or *GDF8* gene) or disease resistance (PRRSV resistance, Bovine tuberculosis resilience, Mannheimia (Pasteurella) haemolytica resilience), (Crispo et al., 2015; Proudfoot et al., 2015; Burkard et al., 2017; Gao et al., 2017; Wang et al., 2017, 2018; Shanthalingam et al., 2016). Thus, genome editing could be a powerful approach to study the functionality of validated genetic markers.

Our results may contribute to the understanding and development of new strategies for improving parasite resistance via selective breeding and marker-assisted selection in Florida Native sheep. Future studies using genome-wide scans to identify other DNA variants associated with parasite resistance and additional animals are necessary to validate our findings before these markers could be used with confidence in a selection and breeding programme. Validated genetic markers associated with parasite resistance could be studied through genome editing tools in future research projects with Florida Native sheep.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2020.11.003.

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