https://doi.org/10.22319/rmcp.v14i1.6077

Article

Identification of candidate genes and SNPs related to cattle temperament using a GWAS analysis coupled with an interacting network analysis

Francisco Alejandro Paredes-Sánchez [a]

Ana María Sifuentes-Rincón [b*]

Edgar Eduardo Lara-Ramírez [c]

Eduardo Casas [d]

Felipe Alonso Rodríguez-Almeida [e]

Elsa Verónica Herrera-Mayorga [f]

Ronald D. Randel [g]

[a] Universidad Autónoma de Tamaulipas, IA-UAMM. Mante, México.

[b] Instituto Politécnico Nacional. Centro de Biotecnología Genómica. Laboratorio de Biotecnología Animal, Blvd. Del Maestro esq. Elías Piña. Col. Narciso Mendoza s/n. Cd. Reynosa, Tam. México.

[c] Instituto Mexicano del Seguro Social, Unidad de Investigación Biomédica de Zacatecas, Zacatecas, México.

[d] United States Department of Agriculture. National Animal Disease Center, Iowa, USA.

[e] Universidad Autónoma de Chihuahua. Facultad de Zootecnia y Ecología, Chihuahua, México.

[f] Universidad Autónoma de Tamaulipas. IBI-UAMM, Mante, México.

[g] Texas A&M University. AgriLife Research. Texas, USA.

*Corresponding author: asifuentes@ipn.mx

Abstract:

The objective of this study was to identify in Angus and Brangus breed animals with extreme temperament, measured as exit velocity, genomic regions and candidate genes associated with bovine temperament. The population was genotyped with the Genomic Profiler HD 150K chip and after the genome-wide association analysis, the SNPs rs133956611 (*P*=2.65 E-06) and rs81144933 (*P*=9.58 E-06) were associated with temperament. The mapping analysis of the regions close to the SNP rs81144933 identified the *SNCA* (alpha-synuclein) and *MMRN1* (multimerin-1) genes at 222.8 and 435.9 Kb downstream respectively, while for the rs133956611 loci the gene *GPRIN3* (GPRIN family-member-3) was identified at 245.7 Kb upstream, all three genes are located on the BTA6 chromosome. The analysis of *SNCA* protein-protein interactions allowed the identification of the genes *APP* (β-amyloid precursor protein), *PARK7* (parkinsonism-associated-deglycase), *UCHL1* (ubiquitin-C-terminal-hydrolase-L1), *PARK2* (parkin-RBR-E3-ubiquitin-protein-ligase), and genes of the *SLC* family as candidates to be associated with bovine temperament. All these candidate genes and their interacting were resequenced, which allowed the discovery of new SNPs in the *SNCA* and *APP* genes. Of these, the SNPs located in introns 5, 8 and 11 of the *APP* gene affect splicing site motifs. These results indicate that *SNCA* and its interacting genes are candidates to be related to bovine temperament.

Key words: Beef cattle, Behaviour, BTA6, Candidate genes, Temperament.

Received: 18/10/2021

Accepted: 16/08/2022

Introduction

Temperament is an economically relevant trait that impacts animal welfare and traits related to productivity. Bovine temperament is considered to be the most important trait of an animal's personality and comprises a wide range of behaviors, from docility to fear and nervousness or a lack of response, attempts to escape, and aggressive behavior, in which various parameters such as general locomotor activity and reactivity to stress are observable. Temperament is affected by age, experience, sex, handling, maternal effects, environmental factors, genetics, species and breed[1,2]. To date, several genomic approaches attempted to

identify genomic regions and genes in which underlying single nucleotide polymorphisms (SNPs) are associated with temperament, a complex phenotypical trait.

Quantitative trait locus (QTL) mapping uncovered the first evidence of genomic regions associated with behavioral traits in dairy breeds[3,4]. The detection of QTLs in the genome led to the proposal of candidate genes under the genomic region encompassed by the QTL, which could potentially be responsible for the differences in trait expression. The identification of candidate genes based on their function and possible involvement in bovine temperament has been a strategy for the search for SNPs. Garza-Brenner *et al*[5] selected a group of 19 genes that participate in the dopamine and serotonin pathway, and through a protein-protein interaction (PPI) analysis, they identified four new interacting candidate genes (*POMC*, *NPY*, *SLC18A2*, and *FOSFBJ*), of which *POMC*, *SLC18A2* and *DRD3*, *HTR2A* (selected based on their function) revealed SNPs associated with Exit Velocity (EV) and Pen Score (PS), which are measurements of bovine temperament in a population of Charolais cattle. The same group found that the variations in these genes (*DRD3*, *HTR2A*, and *POMC)* had an effect on bovine growth (birth weight) in a population of Charolais cattle, showing that the identified variations not only had an effect on bovine temperament but also on live weight traits[6]. Similarly, with the objective of evaluating the potential relationship of two of these SNPs in the *DRD3* and *HTR2A* genes with bovine temperament and growth characteristics, and feed efficiency, a population of Angus, Brangus, and Charolais cattle with temperament assessments was analysed; the results indicated that there was no association with EV and PS, but the SNP in the *HTR2A* gene was associated with feed efficiency in Brangus cattle[7].

Genome-wide association studies (GWAS), based on high-throughput single nucleotide polymorphism (SNP) genotyping technologies, are a relatively recent approach applied to genetic studies of cattle temperament and have allowed the identification of different groups of candidate genes. Lindholm-Perry *et al*[8] analysed a population of the Angus, Hereford, Simmental, Limousin, Charolais, Gelbvieh, and Red Angus breeds to identify genomic regions and genes associated with flight speed (FS); they determined chromosomal regions on BTA 9 and 17 associated and identified within them three genes *GRIA2*, *GLRB*, and *QKI* associated with nearby SNPs. Valente *et al*[9] evaluated a Nellore population using EV to assess their temperament. The *NCKAP5*, *PARK2*, *DOCK1*, *ANTXR1*, *CPE*, and *GUCY1A2* genes were detected as potential candidates for the trait of interest. Finally, Dos Santos *et al*[10] used a Guzerat population in which reactivity was measured as an indicator of temperament. The genes *POU1F1*, *DRD3*, *VWA3A*, *ZBTB20*, *EPHA6*, *SNRPF*, and *NTN4* were proposed as candidate genes responsible for expression of the trait.

In a related context, exome-specific resequencing of specific regions using next-generation sequencing (NGS) technologies has become a powerful technique that allows the identification of SNPs. This method can efficiently capture all variation in the regions of

interest. The potential effects can be assessed in an association study, which provides an effective tool to find SNPs affecting a determined trait[11]. However, due to differences in temperament phenotyping in previous studies, (i.e., each study uses different techniques to assess bovine temperament, pen score, exit velocity, reactivity, which evaluate different aspects of bovine temperament), it is not possible to link information for those genes identified as candidates, or to find a representative biological process, protein-protein interactions between these genes, or a biological path in which these genes converge to visualize how the set of genes explains bovine temperament. Thus, genomic information often remains isolated and needs to be integrated. Hence, the objective was to identify genomic regions and candidate genes associated with temperament in beef cattle through the integration of a GWAS strategy, protein-protein interaction analysis, and SNPs obtained by specific exome resequencing.

Material and methods

Description of animals and biological sample sources

Data and hair samples were obtained from the biobank located at the Animal Biotechnology Laboratory CBG-IPN and were from a cattle population (n= 104) of young Angus (AN, n=63) and Brangus (BR, n=41) bulls, with an average age and body weight of 273 ± 38 d and 272 ± 38 kg, respectively, analysed during two centralized feed efficiency performance tests based on residual feed intake (RFI) in northern Mexico. Data recording and animal management have been previously described by Garza-Brenner *et al*[7]. Briefly, animals were fed in a feedlot for a period of 70 d with a pre-trial adaptation period of 20 d, weighed at the beginning and at the end of the test with intervals of 14 d in which the bovine temperament measurements were made.

From the population, a GWAS was performed using a selective genotyping approach following the strategy of the tails of the phenotypic distribution of bovine temperament measured by exit velocity (EV) because it facilitates the detection of phenotypic differences between alleles[12]. Selective genotyping was achieved by selecting a group of the calmest (n=17; 10-AN and 7-BR) and most temperamental animals (n=17; 9-AN and 8-BR) based on EV values of study population. Temperament was assessed by EV measurements after a stimulus from hair sampling in a chute by measuring the rate of travel over 1.83 m (6 ft) with an infrared sensor (FarmTek Inc., North Wylie, TX, USA). The velocity was calculated as EV= distance (m)/time (s)[13,14]. It was defined the contrasting temperament groups based on

animals' EV measurements. Animals with EV measurements ≤1.9 m/s were classified as calm, and those with EV scores ≥2.4 m/s were classified as temperamental[14,15].

To identify informative SNPs in candidate genes, 91 animals were used. A total of 91 animals were selected as a SNP discovery population: 18 (9 docile; 9 temperamental) of the Angus breed, 68 (44 docile; 24 temperamental) of the Brahman breed, and 5 (2 docile; 3 temperamental) of Charolais breed. From hair samples and ear notches, DNA extraction was performed using the GenElute™ extraction kit (Sigma, St. Louis, Missouri, United States).

GWAS analysis and gene discovery

Thirty-four (34) animals were genotyped using the GeneSeek Genomic Profiler HD 150K chip (Neogen, Lincoln, NE). Association analysis and identification of genomic regions associated with bovine temperament were performed with PLINK 1.9 software[16]. Quality control of the genotypes was performed to identify animals with no assigned genotype or with a low genotyping rate (MIND >0.1). Allele frequency was also evaluated, and those SNPs with lower thresholds (MAF <0.01) were eliminated. Significance threshold was set at $P < 3 \times 10^{-5}$. A Manhattan plot was constructed using qqman: an R package for visualization of GWAS results[17]. Positions of significant SNPs were identified using the bovine *Bos taurus* genome (UMD 3.1.1) and Map Viewer software available at the National Center for Biotechnology Information (NCBI). Genes closest to the significant SNPs (within ~350 kb) were also identified with Map Viewer.

Pathway analysis and protein-protein interactions

For the identification of gene pathways, Gene Ontology (GO) term enrichment and protein-protein interaction (PPI) network analysis were performed in the Ensembl genome browser[18], Gene Ontology database[19], and STRING database[20], respectively.

Candidate genes resequencing

With the objective of identifying SNPs in the coding regions and of the *SNCA* gene and its interacting genes, identified through the protein-protein interaction analysis (PPI), these genes were resequenced in the SNP discovery population. As part of the sequencing strategy,

besides the exons, non-coding regions (140 bp before and after each gene-exon) were also analysed. Thus, a customized panel was designed using the Design Studio software (https://designstudio.illumina.com) (Illumina, San Diego, CA, United States) for the AmpliSeq DNA Gene Assay, in which the coding regions and the boundaries of the *APP*, *PARK7*, *SLC6A2*, *SNCA*, *UCHL1*, *PARK2*, *SLC18A2*, and *POMC* genes were included, using the *Bos taurus* UMD 3.1.1 genome as a reference.

DNA quantification was performed in all steps using the Qubit dsDNA HS Assay kit on the Qubit 3.0 fluorometer (Thermo Scientific, Massachusetts, United States). The libraries were prepared using the reference guide for custom panels AmpliSeq (Document # 1000000036408 v04) of Illumina, following the instructions for 2 pools and for 49–96 pairs of primers per pool. The quality and quantification of the libraries were carried out using the Bioanalyzer 2100 equipment (Agilent, California, United States) with the Agilent DNA 1000 kit. Sequencing (paired-end; read length 126 bp) was performed with the MiniSeq ™ Sequencing System.

Bioinformatics analysis of sequencing data

Sequence reads generated by the MiniSeq™ Sequencing System were aligned with the reference genome UMD 3.1.1 of *Bos taurus* using the Burrows-Wheeler aligner (BWA-MEM) v0.[21]. The reads were processed using Picard v1.135 (http://broadinstitute.github.io/picard) and cleaned by marking and removing duplicate reads to generate BAM files. Variations were identified using the genomic variant call format (GVCF) workflow with HaplotypeCaller[22]. SNPs were generated in VCF files and filtered using the following criteria: variant confidence normalized by depth (QD) <2.0, mapping quality (MQ) <40.0, strand bias (FS) >60.0, HaplotypeScore >13.0, MQRankSum <−12.5, and ReadPosRank-Sum <−8.0[23].

Prediction of the effect of non-coding SNPs on splice sites

To study the effect of the 58 SNPs identified in the non-coding sequences from the exome-specific sequencing of the *SNCA* and *APP* genes, the online ESE finder3.0 web interface (http://krainer01.cshl.edu/cgi-bin/tools/ESE3) was used[24]; the *SNCA* sequences NC_037333.1 and APP: NC_037328.1 were used as input, introducing them intron by intron (<5000 bp) without and with mutations, according to the location of the SNPs. This process allowed to determine if the SNPs were part of a donor (5´) or acceptor (3´) splice site motif;

the programme assigns a score to the input sequence according to the loss of the consensus sequence, so that scores above a default threshold value (donor: 6.67; acceptor: 6.632) are predicted to act as a splice site, allowing the analysis of whether the SNPs affect splice sites motifs.

Results

GWAS analysis and candidate gene identification coupled to protein-protein interaction analysis

Figure 1 depicts a Manhattan plot with the results from the GWAS analysis of SNPs evaluated for their association with temperament in Brangus and Angus cattle. Rs133956611 and rs81144933 were associated with a docile temperament (Table 1). The genes *SNCA* (alpha-synuclein; GenID 282857) and *MMRN1* (multimerin 1; GenID 516574) are located approximately 222.8 and 435.9 Kb upstream respectively, from rs81144933; while the *GPRIN3* (GPRIN family member 3; GenID 517995) gene was identified 245.7 Kb downstream of rs133956611.

Figure 1: Manhattan plot of the -log10 (p-values) for the genome-wide association with exit velocity

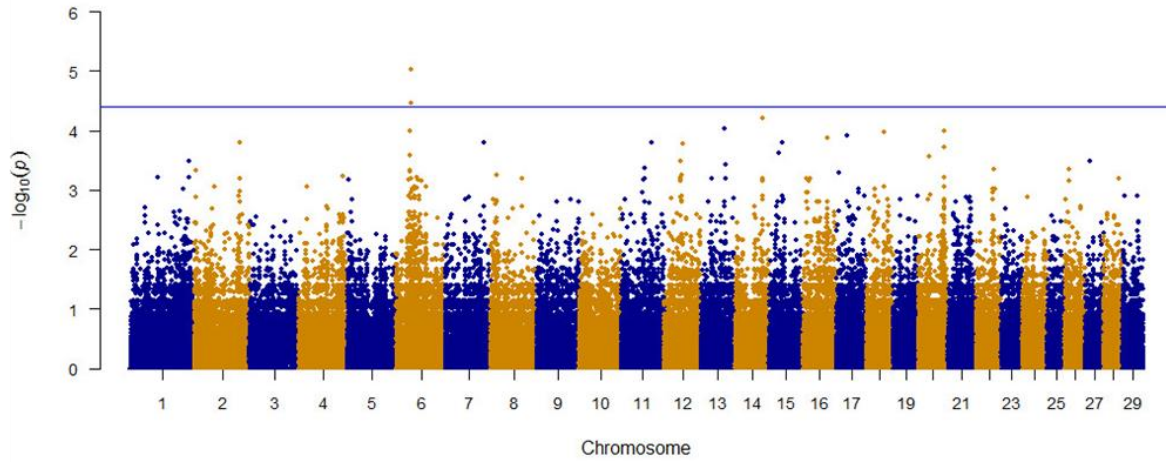


Table 1: SNPs associated with bovine temperament in Angus and Brangus cattle

CHR	rs ID	Position pb	Frecuency T	Frecuency D	*P*-value
6	rs133956611	36,676,986	0.14	0.67	9.2 E-06
6	rs81144933	36,655,249	0.20	0.70	3.48 E-05

T= temperamental; D= docile.

The horizontal line corresponds to a significant threshold of $P=3\times 10^{-5}$ using the identified genes, we proceeded to perform a PPI analysis by querying the STRING[20] database. For *MMRN1*, the PPI analysis showed interactions with genes such as *F5* and *VWF*, involved in the coagulation process (Figure 2), in the Gene Ontology (GO) database, *MMRN1* is annotated with the term GO:0007596, named blood coagulation. For *GPRIN3*, the search engine showed interactions between the phosphorylation process encoded by the *LOC790121* and *OR6N1* genes with proteins that are mainly involved in cytoskeletal assembly and neurotransmission modulation (Figure 3). The GO database showed that this gene was annotated with the term GO:0031175, biological process named neuron projection development, progression of a neuron projection from its formation to the mature structure.

Figure 2: Protein-protein interactions reported for bovine *MMRN1* in the STRING database

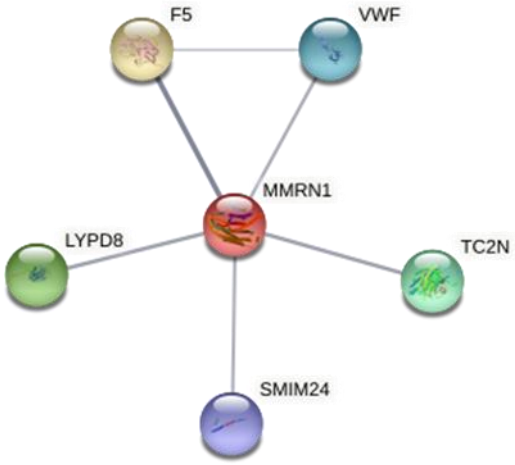
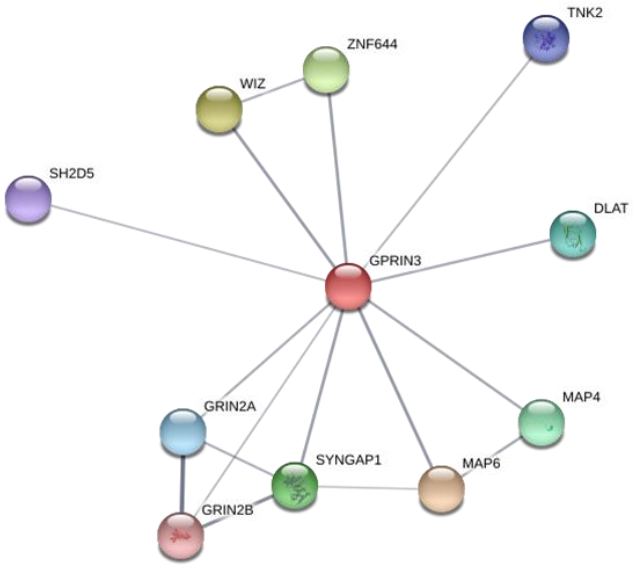


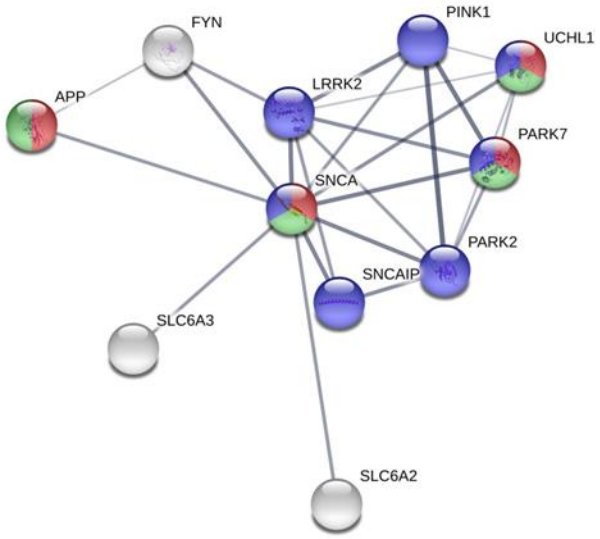
Figure 3: Protein-protein interactions reported for bovine *GPRIN3* in the STRING database



Finally, *SNCA* protein, some GO terms identified (GO:0045920, GO:004241 and GO:0014059) were found to be involved in the regulation, synthesis, and secretion of dopamine. Interestingly, the *SNCA* gene was associated with the terms associated with behavior, including those related to "flight behaviour" and animal responses (through jumping, standing or walking) to internal and external stimuli (terms GO:0007610, GO:0007629 GO:0007628 GO:0007630, respectively).

The PPI analysis indicated that *SNCA* interacts with *APP* (β-amyloid precursor protein), *PARK7* (parkinsonism associated deglycase), and *UCHL1* (ubiquitin C-terminal hydrolase L1) proteins (P= 5.88e-06) involved in adult locomotory behaviour. In addition, the term GO:0008344 reveals strong interactions of *SNCA* with genes belonging to a neurotransmitter transporter family (*SLC6A*) in the network (Figure 4).

Figure 4: Protein-protein interactions reported for bovine *SNCA* in the STRING database



Red nodes annotated with the GO:0008344 term, adult locomotory behavior (p-value 5.88E-06). Green nodes annotated with the GO:0043005 term, neuron projection (p-value 0.000966). Blue nodes annotated with the 05012 KEGG pathway ID, Parkinson s disease (p-value 6.49E-11).

Based on their reported functional role, *GPRIN3* and, particularly, *SNCA* genes could be considered as candidate genes associated with cattle temperament, the *MMRN1* gene analysis indicate no obvious implications for this trait, however its identification could be important for further analysis.

Genetic variation in candidate genes

According to the PPI analysis results, it was inferred that the *APP*, *PARK7*, *SLC6A2*, *UCHL1*, *PARK2*, *SLC18A2*, and *POMC* genes were candidates associated with bovine temperament (Table 2). They were resequenced to discover genetic variation to potentially explain cattle temperament. Fifty-eight (58) SNPs were found in the non-coding regions of the *SNCA* and *APP* genes. Three SNPs were identified in introns 2 and 3 of the *SNCA* gene, and 55 SNPs were identified in introns 1, 5, 8, 11, 13, 14, and 17 of the *APP* gene (Table 3). Fifteen of the 58 SNPs were unique to the Angus breed, 1 in the *SNCA* gene and the remaining in the *APP* gene. The remaining SNPs (n= 43) were informative (polymorphic) in the Brahman and Charolais breeds, as opposed to the Angus breed in which they were uninformative (monomorphic). The allelic frequencies and distribution pattern of the SNPs varied according to the breed.

Table 2: Biological functions and processes associated with interacting *SNCA* genes

Gene	Description
PARK7	No information in cattle. In humans, protects dopaminergic neurons against oxidative damage and degeneration; indirectly inhibits aggregation of α-synuclein[25]; thus, mutations in this gene have been demonstrated to cause Parkinson's disease[26].
SLC6A2	No information in cattle. In humans, controls the action of norepinephrine that support arousal, mood, attention, and reactions to stress; thus, it has been associated with temperamental personality dimensions (novelty seeking, harm avoidance, reward dependence, and persistence)[27].
UCHL1	No information in cattle. In humans, it is abundantly expressed in neurons and interacts with APP, and SNPs in this gene have been implicated in the neurodegenerative disorders Parkinson's disease and Alzheimer's disease[28].
PARK2	In cattle, it has been associated with temperament (flight speed)[9] and in humans in the functions of dopaminergic neurons due to the mutations in this gene associated with Parkinson's disease[29].
SLC18A2	In cattle, it has been associated with temperament (Pen Score) (Garza-Brenner *et al*[5]. It participates in the transport of dopamine, preventing its accumulation and dopaminergic neuron death; therefore, it is a risk factor for Parkinson's disease[30].
POMC	In cattle, it has been associated with temperament (Pen Score)[5]. *POMC* is the precursor for corticotropic hormone (ACTH), which increases the expression of brain-derived neurotrophic factor (BDNF) responsible for neuron proliferation, differentiation, and survival; thus, it has been implicated in Parkinson's disease [31].

From the 58 SNP´s identified in the non-coding regions of *SNCA* and *APP* genes, three SNPs were part of a splice site motif according to established thresholds (donor: 6.67; acceptor: 6.632), as shown in Table 4; the identified SNPs were located in introns 5, 8, and 11. All the splice site motifs were of the acceptor type, that is, they were located at the 3´ end. The SNP g. 9770593 (C/T) did not add or abolish any splice site motif, but only increased the score value, while the SNPs g. 9806689 (G/T) and g. 9845821 (C/G) added and abolished the splice site motifs, respectively.

Discussion

Genomic studies aimed at the exploration of cattle temperament are still scarce, mainly due to the biological complexity of the system, differences in the temperament measurement (objective/subjective), and differences between the studied cattle breeds. In this work, was used the GWAs as an exploratory tool to find candidate genes associated with EV, contrasting by temperament a pool of Angus and Brangus animals. GWAS allowed to identify a genomic region on BTA6 that harbours three candidate genes associated with EV [*SNCA* (Gen ID 282857), *MMRN1* (Gen ID 516574), and *GPRIN3* (Gene ID: 517995)]. For these genes, Chen *et al*[32] reported an elevated expression of *GPRIN3* in the human brain, and information from UniProtKB[33] indicates that the *GPRIN3* protein may be involved in neurite outgrowth. However, the literature data (regarding function and interacting genes) strongly supports the bovine *SNCA* gene as a novel candidate associated with cattle temperament[9,34].

The *SNCA* gene is a highly conserved protein that is abundant in the brain of humans and other species like rats, mice, and monkeys[35]; it is found in neurons, especially in presynaptic terminals[36]. The molecular function of *SNCA* is quite ambiguous, and based on its structure, physical properties, and interacting partners, several hypotheses regarding its normal function in humans have been proposed. For example, it is thought to be involved in the regulation of dopamine release and transport[34]. Consequently, in humans it plays an important role in neurodegenerative disorders. According to Giasson *et al*[37], aggregates of *SNCA* protein in humans cause brain lesions that are characteristic of neurodegenerative synucleinopathies. The *SNCA* gene is associated, in the Kyoto Encyclopedia of Genes and Genomes (KEGG)[38], with biological pathways of neurodegenerative diseases such as Alzheimer's disease (ko05010) and Parkinson's disease (ko05012). Both diseases are important brain disorders in humans. Parkinson's disease is characterized by symptoms related to locomotion (involuntary tremor, muscle stiffness, and postural instability), as well as depression and psychosis, and it involves the progressive loss of dopaminergic neurons, with the main feature presenting as the appearance of inclusion bodies called Lewy bodies, the main component of which is *SNCA*[37].

Although the pathological alterations linked to those human diseases cannot be extrapolated to this study model, this biological link provides some evidence to support the findings because the understanding of the relationship between genotype and phenotype in humans was derived from model animals with mutations in orthologous genes. Large animal species, such as dog, pig, sheep and cattle, have been some of the most important model animals, mainly because they are more similar to humans than mice (similar size, genetics, and physiology). Thus, discoveries in humans can serve as a reference to infer effects on bovine temperament[39].

Connecting gene networks to explain cattle temperament

Despite scarce attempts to identify genes and genomic regions underlying the genetic architecture of temperament, until now there have been no reports connecting the gene networks associated with this complex trait.

Protein-protein interaction analysis of the *SNCA* gene allowed to identify and analyse six additional genes, of which two gene members of the *SLC* family (*SLC18A2* and *SLC6A4*) have already been identified by Garza-Brenner *et al*[5] as interacting genes in a protein-protein network based on dopamine- and serotonin-related genes. These authors also found a SNP located in the *SLC18A2* gene that causes a change in the amino acid sequence from alanine to threonine, with significant effects on temperament as measured by pen scores. In addition, the PPI analysis included genes in the *PARK* family (*PARK2* and *PARK7*), which encode ubiquitin ligase proteins, including parkin RBR E3. The gene *PARK2* was identified by Valente *et al*[9] as a candidate gene associated with temperament in Nellore cattle; the authors used EV as a test to evaluate bovine temperament. Multiple studies have used the GWAS strategy to identify genes that are linked to bovine temperament phenotypes[8-10], but in none of these cases has it been possible to establish interactions between the identified genes, and the information from each study seems to be isolated and independent, preventing the clarification of the genetic architecture of temperament from the information available to date. In addition, the set of candidate genes does not seem to be associated with a representative biological process that suggests participation in temperament. The identification of *SNCA* in this work allows to connect the results of Valente *et al*[9] and Garza-Brenner *et al*[5], showing that the genes identified through different strategies (GWAS and protein-protein interaction network analysis) present an important connection. According to these results, it was explored the genetic variation in these genes in cattle with an emphasis on their coding sequences, and the results revealed a high conservation of the exonic sequences in all seven analysed genes. In humans, a low genetic variation has been reported between genes such as *SNCA* and *UCHL1*[40].

Interestingly, and according to previous reports, a high genetic variation was found in the in the non-coding regions of the bovine *SNCA* and *APP* genes.

The exact function of the amyloid beta (A4) precursor protein (*APP)* gene is unknown, but it has been associated with meat softness in pigs[41], can participate in the formation of neurons, and is known for its participation in Alzheimer's disease[42]. Because patients with Alzheimer's disease show the presence and accumulation of both *SNCA* and *APP* proteins, it has been proposed that they may be related in some way. Roberts *et al*[43] have shown that *SNCA* overexpression increases *APP* levels, and certain mutations in *SNCA* increase the

processing of *APP*, so the discovery of mutations in the coding regions of these genes could have a functional impact on them and therefore on bovine temperament.

It has been documented that approximately 21% of bovine genes are alternatively spliced[44]. *In silico* analyis identified three *APP*-SNP´s with the potential to have a functional effect in the pre-mRNA splicing process and, therefore, the expression of bovine temperament. As far as known, no different isoforms of the bovine *APP* gene have been reported, but splice site motifs in bovine genes have been reported to be highly conserved relative to humans[44]. The human and bovine genes for *APP* are orthologs, having the same number of amino acids (770) and an identical amino acidic sequence. In humans, 8 different isoforms of the APP gene have been identified due to the alternative splicing in exons 7, 8, and 15, which terminates *APP* gene expression in neurons, resulting in the implication of a fundamental role in Alzheimer's disease[45]. Here there was identified 3 SNPs that affect, add, and abolish splice site motifs in the *APP* gene, in introns 5, 8, and 11, so they could probably affect the final product and have an effect on the expression of bovine temperament.

In the present study, it was used the contrasting phenotype strategy to perform an exploratory GWAS analysis to identify candidate genes for temperament in cattle, and even with the small sample size limitation, the results showing a connection between SNCA and temperament are consistent with larger GWAS studies. Additionally, the coupling of these result with a PPI analysis allowed to establish connections between different genes that were previously identified within the association to the locomotor system. Fine mapping of the candidate genes predicted that the GWAS and PPI genes confirmed the existence of SNPs with the potential to affect bovine temperament. The present study provides valuable information that contributes to the -still scarce- efforts to describe the cattle temperament genetic architecture, and shows that an analytic strategy is appropriate for application in studies with a limited sample size, especially in countries where phenotyping for this complex trait is limited.

Conclusions and implications

A BTA6 genomic region (36,655,249-36,676,986 bp) neighboring the *SNCA* gene was associated with temperament trait in Angus and Brangus breeds. Six genes, linked to SNCA, were identified as being potentially associated with temperament. From those, the *APP* gene harboured three SNPs with a potential effect on the pre-mRNA splicing process and expression of bovine temperament.

Acknowledgements

This investigation was funded by research grants supported by CONACYT and IPN (project 294826, SIP 20171674) and partial financial support by CONARGEN, A.C. to support the feeding performance tests. The authors would also like to acknowledge the different herd owners and the technical staff from the Palomas complex UGRCH, who collected and provided the data and samples used in this study. The mention of trade name, proprietary products, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval for the exclusion of other products that may be suitable. The USDA is an Equal Opportunity Employer.

Literature cited:

1. Buchenauer D. Genetics of behaviour in cattle. In: Fries R Ruvinsky A, editors. The genetics of cattle. CABI Publishing, Wallingford;1999:365–390.

2. Mormède P. Molecular genetics of behaviour: research strategies and perspectives for animal production. Livest Prod Sci 2005;93(1):15–21.

3. Schmutz SM, Stookey JM, Winkelman-Sim DC, Waltz CS, Plante Y, Buchanan FC. A QTL study of cattle behavioural traits in embryo transfer families. J Hered 2001; 92(3):290–292.

4. Hiendleder S, Thomsen H, Reinsch N, Bennewitz J, Leyhe-Horn B, Looft C, *et al*. Mapping of QTL for body conformation and behavior in cattle. J Hered 2003; 94(6):496–506.

5. Garza-Brenner E, Sifuentes-Rincón AM, Randel RD, Paredes-Sánchez FA, Parra-Bracamonte GM, Arellano-Vera W, *et al*. Association of SNPs in dopamine- and serotonin-pathway genes and their interacting genes with temperament traits in Charolais cows. J Appl Genetics 2016;58(3):363–371.

6. Garza-Brenner E, Sifuentes-Rincón AM, Rodríguez-Almeida FA, Randel RD, Parra-Bracamonte GM, Arellano-Vera W. Influence of temperament-related genes on live weight traits of Charolais cows. R Bras Zootec 2020:49:e20180121.

7. Garza-Brenner E, Sifuentes-Rincón AM, Rodríguez Almeida FA, Randel RD, Parra-Bracamonte GM, Arellano Vera W. Influence of genetic markers on the feeding behavior of yearling bulls. Rev Colomb Cienc Pecu 2019;32(1):14-20.

8. Lindholm-Perry AK, Kuehn LA, Freetly HC, Snelling WM. Genetic markers that influence feed efficiency phenotypes also affect cattle temperament as measured by flight speed. Anim Genet 2014;46(1):60-64.

9. Valente TS, Baldi F, Sant´Anna AC, Albuquerque LG, Da Costa MJRP. Genome-wide association study between single nucletide polymorphismos and flight speed in Nellore cattle. PLoS One 2016;11(6):1-18.

10. Dos Santos FC, Campolina PMG, De Souza PA, Ávila PMF, Ventura RV, Rosse IC, *et al*. Identification of candidate genes for reactivity in Guzerat (*Bos indicus*) cattle: a Genome-Wide Association Study. Plos One 2017;12(1):1-15.

11. Jiang L, Liu X, Yang J, Wang H, Jiang J, Liu L, *et al*. Targeted resequencing of GWAS loci reveals novel genetic variants for milk production traits. BMC Genomics 2014;15(1):1105.

12. Kurz JP, Yang Z, Weiss RB, Wilson DJ, Rood KA, Liu GE, Wang Z. A genome-wide association study for mastitis resistance in phenotypically well-characterized Holstein dairy cattle using a selective genotyping approach. Immunogenetics 2019;71(1):35-47.

13. Burrow HM. Measurements of temperament and their relationships with performance traits of beef cattle. Anim Breed 1997;65(7):477–495.

14. Curley KOJr, Paschal JC, Welsh THJr, Randel RD. Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J Anim Sci 2006;84(11):3100-3103.

15. Burrow HM. Effect of intensive handling of zebu crossbred weaner calves on temperament. Proc Assoc Advmt Anim Breed Genet 1991;9:208-211.

16. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;8(3):559-575.

17. Turner SD. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv 2014;3(25):005165.

18. Zerbino DR, Achuthan P, Akanni W, Amode MR, Barrell D, Bhai J. Ensembl 2018. Nucleic Acids Res 2018;46(D1):754–761.

19. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, Thomas PD. PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. Nucleic Acids Res 2019;47(D1):D419-D426.

20. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, *et al*. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017;45(D1):362-368.

21. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 2009;25(14):1754–1760.

22. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, LevyMoonshine A, *et al*. From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. Curr Protoc Bioinf 2013;43(1110):11101–111033.

23. Choi JW, Liao X, Stothard P, Chung WH, Jeon HJ, Miller SP, *et al*. Whole-genome analyses of Korean native and Holstein cattle breeds by massively parallel sequencing. PLoS One 2014;9(7):e101127.

24. Cartegni L, Wang J, Zhu Z, Zhang MQ, Krainer AR. ESEfinder: A web resource to identify exonic splicing enhancers. Nucleic Acid Res 2003;31(13):3568-3571.

25. Dolgacheva LP, Berezhnov AV, Fedotova EI, *et al*. Role of DJ-1 in the mechanism of pathogenesis of Parkinson's disease. J Bioenerg Biomembr 2019;51(3):175–188.

26. He L, Lin S, Pan H, Shen R, Wang M, Liu Z, *et al*. Lack of association between DJ-1 gene promoter polymorphism and the risk of parkinson's disease. Front Aging Neurosci 2019;11(24):1-11.

27. Narita SI, Kazuhiko N, Kenta N, Maki Y, Eiji O, Nobuyo I. Analysis of association between norepinephrine transporter gene polymorphisms and personality traits of NEO-FFI in a Japanese population. Psychiatry Investig 2015;12(3):381-387.

28. Rydning SL, Backe PH, Sousa MML, Iqbal Z, Øye AM, Sheng Y, *et al.* Novel UCHL1 mutations reveal new insights into ubiquitin processing. Hum Mol Genet 2017;26(6):1031-1040.

29. Bakhit YH, Ibrahim MO, Amin M, Mirghani YA, Hassan MA. *In silico* analysis of SNPs in PARK2 and PINK1 genes that potentially cause autosomal recessive Parkinson disease. Adv Bioinformatics 2016;9313746:1-5.

30. Brighina L, Riva C, Bertola F, Saracchi E, Fermi S, Goldwurm S, *et al*. Analysis of vesicular monoamine transporter 2 polymorphisms in Parkinson's disease. Neurobiol Aging 2013;34(6):1712.e9-13.

31. Shadrina M, Nikopensius T, Slominsky P, Illarioshkin S, Bagyeva G, Markova E, *et al*. Association study of sporadic Parkinson's disease genetic risk factors in patients from Russia by APEX technology. Neurosci Lett 2006;405(3):212-6.

32. Chen LT, Gilman AG, Kozasa T. A Candidate target for G protein action in brain. J Biol Chem 1999;274(38):26931–26938.

33. Chen C, Huang H, Wu CH. Protein bioinformatics databases and resources. Methods Mol Biol 2017;1558:3-39.

34. Siddiqui IJ, Pervaiz N, Abbasi AA. The Parkinson disease gene SNCA: Evolutionary and structural insights with pathological implication. Sci Rep 2016;6(1):24475.

35. Deng H, Yuan L. Genetic variants and animal models in SNCA and Parkinson disease. Ageing Res Rev 2014;15:161-76.

36. Campêlo CLDC, Silva RH. Genetic variants in SNCA and the risk of sporadic Parkinson's disease and clinical outcomes: A review. Parkinsons Dis 2017;4318416:1-11.

37. Giasson BI, Duda JE, Murray IVJ, Chen Q, Souza JM, Hurtig HI, *et al*. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science 2000;290(5493):985-989.

38. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: New perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 2017;45(D1):353-361.

39. Pinnapureddy AR, Stayner C, McEwan J, Baddeley O, Forman J, Eccles MR. Large animal models of rare genetic disorders: sheep as phenotypically relevant models of human genetic disease. Orphanet J Rare Dis 2015;10:107-115.

40. Dong N, Zhang X, Liu Q. Identification of therapeutic targets for Parkinson's disease via bioinformatics analysis. Mol Med Rep 2017;15(2):731-735.

41. Lobjois V, Liaubet L, San Cristobal M, Glénisson J, Fève K, Rallières J. A muscle transcriptome analysis identifies positional candidate genes for a complex trait in pig. Anim Genet 2008;39(2):147-162.

42. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci USA 1985;82(12):4245-4249.

43. Roberts HL, Schneider BL, Brown DR. α-Synuclein increases β-amyloid secretion by promoting β-/γ-secretase processing of APP. PLoS One 2017;12(2):e0171925.

44. Chacko E, Ranganathan S. Genome-wide analysis of alternative splicing in cow: Implications in bovine as a model for human diseases. BMC Genomics 2009;10(3):S11.

45. Sandbrink R, Masters CL, Beyreuther K. APP gene family. Alternative splicing generates functionally related isoforms. Ann N Y Acad Sci 1996;777:281-287.

Table 3: SNPs identified by specific exome sequencing in each population of *APP, PARK7, SLC6A2*, *SNCA, UCHL1, PARK2, SLC18A2,* and *POMC* genes

Gene	Position (bp)	Region	Alleles		Frecuency					
					Angus		Brahman		Charolais	
			Ref	Alt	Ref	Alt	Ref	Alt	Ref	Alt
	36297353	Intron 3	G	A			0.9924	0.0076	1.0	0.0
SNCA	36297374	Intron 3	A	G			0.8500	0.1500	1.0	0.0
	36297422 ¥	Intron 2	T	A	0.5000	0.5000				
	9674371	Intron 1	T	C			0.9717	0.0283	1.0	0.0
	9674423	Intron 1	A	C			0.9403	0.0597	1.0	0.0
	9674429	Intron 1	T	A			0.9478	0.0522	1.0	0.0
	9674430 ¥	Intron 1	T	A	0.9722	0.0278				
	9674431*	Intron 1	A	T	0.9706	0.0294	0.9925	0.0075	1.0	0.0
	9674437	Intron 1	T	C			1.0000	0.0	0.9000	0.1000
	9674448	Intron 1	T	C			0.9921	0.0079	1.0	0.0
	9674451	Intron 1	A	G			0.9921	0.0079	0.9000	0.1000
	9674455*	Intron 1	G	A	0.6071	0.3929	0.0093	0.9907	0.5000	0.5000
	9770586*	Intron 5	A	G/T	0.6944	0.3056/0.0	0.8772	0.0395/0.0833	0.8000	0.2000/0.0
	9770593	Intron 5	C	T			0.3507	0.6493	1.0	0.0
	9770633	Intron 5	G	A			0.5373	0.4627	1.0	0.0
APP	9803985*	Intron 8	C	T	0.9722	0.0278	0.0944	0.9056	1.0	0.0
	9803991*	Intron 8	A	G	0.9722	0.0278	0.0909	0.9091	1.0	0.0
	9806624*	Intron 8	A	G	0.9167	0.0833	0.0574	0.9426	0.8000	0.2000
	9806672	Intron 8	T	C			0.9769	0.0231	0.8000	0.2000
	9806689	Intron 8	G	T			0.9851	0.0149	1.0	0.0
	9845631	Intron 11	C	A			1.0000	0.0000	0.8000	0.2000
	9845821	Intron 11	C	G			0.7177	0.2823	1.0	0.0
	9845862 ¥	Intron 11	G	T	0.8750	0.1250				
	9845934	Intron 11	G	A			0.9844	0.0156	1.0	0.0
	9845944	Intron 11	G	A			0.8750	0.1250	1.0	0.0
	9845966	Intron 11	G	A			0.9692	0.0308	1.0	0.0
	9845980	Intron 11	A	G			0.8056	0.1944	1.0	0.0
	9863873*	Intron 13	T	C	0.9722	0.0278	0.0522	0.9478	1.0	0.0
	9863960	Intron 13	T	C			0.0818	0.9182	1.0	0.0

9863974¥	Intron 13	T	C	0.6666	0.3333				
9863983¥	Intron 13	T	C	0.0	1.0				
9863984¥	Intron 13	G	T	0.0	1.0				
9866489	Intron 13	G	A			0.8433	0.1567	1.0	0.0
9866528	Intron 13	A	G			0.9925	0.0075	1.0	0.0
9866542¥	Intron 13	A	G	0.9722	0.02				
9866545	Intron 13	C	T			0.8433	0.1567	1.0	0.0
9866552¥	Intron 13	T	C	0.9118	0.08				
9866569	Intron 13	C	T			0.8624	0.1376	1.0	0.0
9879860	Intron 13	T	C			0.7881	0.2119	1.0	0.0
9880018	Intron 13	C	A			0.5694	0.4306	1.0	0.0
9880025	Intron 13	G	T			0.7787	0.2213	1.0	0.0
9889605¥	Intron 14	C	G	0.6250	0.3750				
9889627	Intron 14	G	A			0.9462	0.0538	1.0	0.0
9889677*	Intron 14	G	T	0.0	1.0	0.9925	0.0075	0.0	1.0
9889687¥	Intron 14	T	C	0.4167	0.5833				
9891054¥	Intron 14	C	T	0.5833	0.4167				
9891056¥	Intron 14	T	C	0.5000	0.5000				
9891124¥	Intron 14	G	T	0.4000	0.6000				
9891130¥	Intron 14	A	G	0.4063	0.5938				
9891155¥	Intron 14	T	G	0.4063	0.5938				
9918483	Intron 17	C	T			0.9841	0.0159	1.0	0.0
9918506*	Intron 17	A	G	0.1389	0.8611	0.9250	0.0750	0.2000	0.8000
9918508	Intron 17	C	T			0.9655	0.0345	1.0	0.0
9918512	Intron 17	C	T			0.9914	0.0086	1.0	0.0
9931517	Intron 17	C	G			0.9924	0.0076	1.0	0.0
9931524	Intron 17	C	G			0.9924	0.0076	1.0	0.0
9931525	Intron 17	T	G			0.9924	0.0076	1.0	0.0
9931529	Intron 17	C	T			1.0000	0.0000	0.9000	0.1000

* Variations present in the three populations. ¥ Specific variations in the Angus population.

Table 4: The ESE finder results for non-coding SNPs identified in the *SNCA* and *APP* genes

Gene	Position	Intron	SNP		Site Sequence	Donor/ acceptor	Score
APP	9770593	5	C	WT	CT**C**TCCCCTCGTCAGTGCTGTAGTTCAGGT	acceptor	6.74720
			T	M	CT**T**TCCCCTCGTCAGTGCTGTAGTTCAGGT	acceptor	7.11480 ↑
	9806689	8	G	WT	------	------	------
			T	M	CTT**T**GGATTTGCCAGGCACACTCACCTCCA	acceptor	6.81380 ↑
	9845821	11	C	WT	CTCCTTCCACAACAGAAGG**C**GCTATTTTAA	acceptor	6.71530
			G	M	------	------	------

The SNP nucleotide is highlighted in bold in the sequence. WT: wild type. M: sequence with non-coding SNP. ↑ indicates an increased score compared with the wild type sequence.