

Association of SNPs in dopamine and serotonin pathway genes and their interacting genes with temperament traits in Charolais cows

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Abstract Cattle temperament is a complex trait, and molecular studies aimed at defining this trait are scarce. We used an interaction networks approach to identify new genes (interacting genes) and to estimate their effects and those of 19 dopamine- and serotonin-related genes on the temperament traits of Charolais cattle. The genes proopiomelanocortin

(*POMC*), neuropeptide Y (*NPY*), solute carrier family 18, member 2 (*SLC18A2*) and FBJ murine osteosarcoma viral oncogene homologue (*FOSFBJ*) were identified as new candidates. Their potential to be associated with temperament was estimated according to their reported biological activities, which included interactions with neural activity, receptor function, targeting or synthesis of neurotransmitters and association with behaviour. Pen score (PS) and exit velocity (EV) measures were determined from 412 Charolais cows to calculate their temperament score (TS). Based on the TS, calm ($n = 55$; TS, 1.09 ± 0.33) and temperamental ($n = 58$; TS, 2.27 ± 0.639) cows were selected and genotyped using a 248 single-nucleotide variation (SNV) panel. Of the 248 variations in the panel, only 151 were confirmed to be polymorphic (single-nucleotide polymorphisms; SNPs) in the tested population. Single-marker association analyses between genotypes and temperament measures (EV, PS and/or TS) indicated significant associations of six SNPs from four candidate genes. The markers rs109576799 and rs43696138, located in the *DRD3* and *HTR2A* genes, respectively, were significantly associated with both EV and TS traits. Four markers, rs110365063 and rs137756569 from the *POMC* gene and rs110365063 and rs135155082 located in *SLC18A2* and *DRD2*, respectively, were associated with PS. The variant rs110365063 located in bovine *SLC18A2* causes a change in the amino acid sequence from Ala to Thr. Further studies are needed to confirm the association of genetic profile with cattle temperament; however, our study represents important progress in understanding the regulation of cattle temperament by different genes with divergent functions.

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Implications The serotonin and dopamine signalling systems are essential for different behavioural phenotypes in both invertebrates and vertebrates. Studies focused on the genetic basis of behaviour in cattle are scarce. As enough evidence supports the importance of behavioural traits on the productivity of beef cattle of different breeds, the identification of intrinsic genetic sources of variation could be of fundamental application by facilitating the selection of appropriate temperament genotypes. Selecting livestock with greater docility has positive benefits for improving beef cattle performance, in addition to improving human safety and animal welfare.

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Introduction

Basic behavioural traits of cattle include social behaviours such as aggression and temperament (Buchenauer 1999). These traits may have a direct economic value and could be included in selection strategies (Mormède 2005; Nkrumah et al. 2007). Studies associating behaviour with production traits have found that nervous or aggressive animals have decreased milk flow and yield (Rushen, cited by Hiendleder et al. 2003). Temperament, defined as the animal's response to handling by humans (Burrow and Dillon 1997), has been studied in different breeds and affects growth, health, performance and carcass merit (Nkrumah et al. 2007; Cooke 2014).

At the molecular level, behavioural traits in cattle have been assessed using different approaches. Schmutz et al. (2001) reported the identification of quantitative trait loci (QTL) for response to isolation in beef cattle, while Hiendleder et al. (2003) reported the discovery of QTL for milking speed. These studies uncovered the first evidence of QTL associated with behavioural traits in dairy breeds. Genome-wide association studies (GWAS) to study temperament and meat quality in Nellore–Angus beef cattle found an association between response to social separation in a pen and a gene regulating sodium ion transport, indicating a difference in the responsiveness of the nervous system (Hulsman Hanna et al. 2014).

Due to the influence of behavioural trait genes, the transporters and receptors of the serotonin and dopamine signalling pathways have been considered to harbour genetic variations that may be associated with variable behavioural responses (Momozawa et al. 2005).

The serotonin and dopamine signalling systems are central to behavioural phenotypes in both invertebrates and vertebrates. In fact, defects in the noradrenergic system have been implicated in many mood, cognitive and neurological disorders related to abnormal social behaviour (Våge and Lingaas 2008). In cattle, Lühken et al. (2010) studied the genetic variation affecting the enzyme monoamine oxidase A (*MAOA*) and reported five single-nucleotide polymorphisms (SNPs), one of which, located in exon XV, was a non-synonymous mutation. In silico analysis did not show consistent results regarding the functional impact, and non-significant associations were found between this *MAOA* polymorphism and the behaviour-related scores analysed in the study. Glenske et al. (2011) found an association between an SNP located in an intron of the dopamine receptor gene (*DRD4*) and performance in a docility test. *DRD4* is involved in curiosity and novelty seeking in mammals (Rubinstein et al. 1997).

Molecular studies supporting the genetic control of cattle temperament may lead to applications in production and animal welfare. Upon comparison of the different strategies employed, the candidate gene approach appears to be the most direct and effective; however, it is limited by the lack of a deep understanding of the molecular pathways involved in the genetic

variability affecting these traits (Mormède 2005). The search for molecular associations using the candidate gene approach may be improved by coupling different search strategies, such as interaction networks analyses, i.e. protein–protein interactions (PPIs) and the guilt-by-association strategy. The guilt-by-association strategy uses the biological information available in databases and statistical methods to identify potential candidate genes in silico (Paredes-Sánchez et al. 2015). An advantage of this methodology is that it is not based exclusively on the biological information from the focal species; it actually uses all of the reference information available from other species, especially from better-understood model species, such as humans, mice and *Drosophila* (Mormède 2005).

In this study, we used an interaction networks approach to identify and prioritise new genes (interacting genes) and to estimate their effects and those of 19 dopamine- and serotonin-related genes on the temperament traits of Charolais cows.

Materials and methods

Data from 412 Charolais cows were included in the study. All were born between 2004 and 2013 and were managed in four herds located in Sonora (herd 1, $n = 50$), Tamaulipas (herd 2, $n = 77$) and Nuevo Leon (herd 3, $n = 145$ and herd 4, $n = 140$), Mexico. Animals from each herd were grouped by age into young cows (2–3 years of age) and mature cows (>4 years of age). Similar management objectives in the four herds were focused on breeding purebred Charolais cattle and the sale of breeding stock.

Temperament was assessed using pen score (PS) and exit velocity (EV). For PS, three evaluators assigned a score on a five-point scale, where 1 = unalarmed and unexcited animal that walks slowly away from the evaluator and 5 = very excited and moved aggressively toward the technician in a manner that requires evasive action to avoid contact between the technician and the cow, as described by Hammond et al. (1996). EV was assessed following the stimulus of hair sampling in the chute by measuring the rate of travel over a 1.83-m distance with an infrared sensor (FarmTek, Inc., North Wylie, TX, USA). The velocity was calculated as $EV = \text{distance (m)}/\text{time (s)}$. Individual temperament scores (TS) were calculated by averaging the PS and EV [$TS = (PS + EV/2)$]. PS are considered subjective measures of temperament, while EV is considered an objective measurement (Norris et al. 2014). The combined score utilised multiple assessments (i.e. PS and EV) and may allow a more accurate classification of temperament (Burdick et al. 2011).

As cattle temperament is influenced by age and handling management, among other environmental factors, animals were classified into three groups, calm, intermediate and temperamental, in each herd based on the herd-group average values $(TS) \pm 1$ standard deviation (SD).

To examine the temperament differences of the segregated groups in relation to herd and age group factors, a linear model including the fixed effect of the interaction of the temperamental group with herd and the effects of the interactions of the temperament and age group was fitted. After this analysis, a least squares means comparison was performed. SAS 9.0 (SAS Statistical Analysis System, SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Identification and prioritisation of candidate genes influencing temperament

A strategy for the identification of interacting genes was implemented using the BosNet approach. A list of reference genes was determined in accordance with the BosNet user instructions (<http://www.cbg.ipn.mx/Investigacion/Paginas/BosNet.aspx>). A literature review was conducted to select the reference genes for temperament reported to be involved in the dopaminergic and serotonergic pathways in humans, horses and dogs (Sifuentes-Rincón et al. 2016). These candidates included all of the genes of the dopamine and serotonin pathways in cattle with reported sequences in NCBI resources (<http://www.ncbi.nlm.nih.gov/>; Bovine Genome <http://www.bovinegenome.org/>; *DRD1*, gene ID: 281125; *DRD2*, gene ID: 281126; *DRD3*, gene ID: 537043; *DRD5*, gene ID: 526221; *DDC*, gene ID: 280762; *HTT*, gene ID: 615059; *HTR1A*, gene ID: 407137; *HTR1B*, gene ID: 317707; *HTR2A*, gene ID: 407230; *TDO2*, gene ID: 530397; *TH*, gene ID: 280707; *DBH*, gene ID: 280758; *ADRA2A*, gene ID: 282135; *ADRA2B*, gene ID: 516422; *NET*, gene ID: 282363; *PNMT*, gene ID: 281413; *COMT*, gene ID: 618278; *MAOA*, gene ID: 281293; *MAOB*, gene ID: 338445; *TPH1*, gene ID: 781941; and *TPH2*, gene ID: 100336620).

To identify and prioritise candidate genes for each of the integrated networks, the interactions of the reference genes were extracted and the degree of association with temperament (DAT) was calculated for each gene in the following subnet.

$$DAT = \sum_{j \in \text{ref genes}} W_{ij} \cdot \sum_{j \in \text{ref genes}} P_{ij}$$

where W_{ij} is the linkage temperament connecting protein i and reference protein j and P_{ij} is the number of links connecting protein i and reference protein j (excluding itself). Thus, the probability that each of these proteins is associated with temperament or behaviour traits was evaluated based on its interaction with genes known to be functionally associated with this trait.

Using this information, the predictive power of each of the modelled networks for *Bos taurus* was evaluated and the capacity of these networks to correctly identify genes associated with temperament was measured. This predictive power was characterised using receiver operating characteristic (ROC) curves. The area under the curve (AUC) was used as an

indicator of the predictive power. AUC values ≤ 0.5 represent random predictions. AUC values > 0.5 represent predictions ranging from average to good.

For the selection of interacting candidate genes for temperament, the new score was used to calculate the positive predictive value (PPV), which indicates the likelihood of an association between the gene and the target trait (Paredes-Sánchez et al. 2015).

Genotyping and association analysis of selected SNPs affecting temperament

DNA was isolated from hair samples and genotyped using the Sequenom MassARRAY® platform (GeneSeek, Inc., Lincoln, NE, USA). The genotypic and allelic frequencies were estimated using Genepop® 4.0.10 software (Rousset 2008).

Based on availability in the GenBank databases, we consider to include a 248 single-nucleotide variation (SNV) panel. These markers were defined as SNVs, with the exception of a few markers located at the *DRD4*, *TH* and *DBH* genes, as no frequency data in any population was available to be considered as an SNP. The included SNVs were located mainly in the coding regions (71%) of 19 reference genes and four of their five interacting genes. The SNVs were represented by 48.27% synonymous and 23% non-synonymous variants. As expected, the number of SNVs included from each gene was different: the highest was 35 SNVs from the bovine *HTT* gene and the lowest was one SNP in the *DRD4* gene. After genotyping and considering the frequencies (minor allele frequency $> 10\%$), we defined them as SNPs.

The effects of selected SNP genotypes on the PS, EV and TS were assessed from the selected animals dataset using a general linear model procedure that included the fixed effects of herd, age group, year of birth and genotype in each studied SNP. In the model, $Y_{ijk} = \mu + HD_i + GE_j + G_k + \varepsilon_{ijk}$, where Y (EV, PS and TS) represents the dependent studies traits, HD is the i th herd effect (Herd 1... Herd 4), GE is the j th age group effect (young and mature cows), G is the effect of the k th genotype in each individual SNP and ε is the random error. Least squares means of genotypes were estimated for the SNPs that demonstrated significant effects ($p < 0.05$) and a comparison of the means was performed with the PDIFF statement. All procedures were performed using SAS 9.0 software.

Results

Temperament assessment and contrasting population examination

The distribution of animals within each herd classified as calm, intermediate and temperamental is presented in Table 1. Both interactions between temperament classification

Table 1 Distribution of cows from the four herds segregated into three temperament classifications

Herd	Temperament classification			Total
	Calm	Intermediate	Temperamental	
Herd 1	6	37	7	50
Herd 2	13	53	11	77
Herd 3	19	106	20	145
Herd 4	17	103	20	140
Total	55	299	58	412

were significant ($p = 0.0001$), indicating differences in the classification categories among the studied herds and age groups.

In general, a gradient of greater temperament traits was observed in the young cow grouping compared with that in the older cows. Nonetheless, no significant differences were observed in the means for EV between the animals classified as calm in both age groups ($p = 0.068$). PS and TS showed similar patterns, with greater means for young cows in the temperamental groups ($p \leq 0.04$) in comparison with the other levels of interaction, even with temperamental old cows (data not shown).

For all traits, the interactions between the temperamental groups and herd were significant ($p < 0.0001$), and the estimated means are illustrated in Fig. 1. The cows in herd 4 showed greater differences in the assessment of EV, which was particularly evident in the cows belonging to the temperamental category, in relation to the intermediate and temperamental categories compared with those from the other herds analysed; additionally, herd 4 had the highest EV (3.68 ± 0.12 m/s).

For PS, larger differences were observed in the temperamental cows of the different herds. Herd 1 had the largest mean EV (3.07 ± 0.14 points), while the intermediate and calm animals showed more equilibrated means among the herds, producing compact groups (Fig. 1b).

For TS, the mean values showing the interaction between the temperament classification and herd are depicted in Fig. 1c. Similar to the pattern observed for EV, herd 4 exhibited a larger mean TS compared with the other herds in the same temperament classification ($p < 0.0001$); similarly, the calm groups of the herds showed more similarities in the mean TS (Fig. 1c).

Association of SNPs located in the candidate genes with temperament

The selection criterion for the new interacting candidate genes to be associated with temperament was a PPV greater than 0.5 (genes with a greater than 50% probability). As shown in Fig. 2, the BosNet-prioritised genes included *POMC*

(proopiomelanocortin, gene ID: 504216), *NPY* (neuropeptide Y, gene ID: 504216), *SLC18A2* [solute carrier family 18 (vesicular monoamine transporter), member 2, gene ID: 282471], *SLC6A4* [solute carrier family 6 (neurotransmitter transporter), member 4, gene ID: 282365] and *FOSFBJ* (murine osteosarcoma viral oncogene homologue; gene ID: 280795); all genes showed PPVs of 0.58.

Animals from the four herds [$n = 58$ with the highest TS (temperamental group), $n = 55$ with the lowest TS (calm group)] were selected for the genotyping and association analyses.

From the tested panel, only 151 were confirmed to be polymorphic (SNPs) in the tested population (minor allele frequency $> 10\%$).

Table 2 shows the results of the association analysis. Six SNPs from four candidate genes were associated with one or more temperament traits (EV, PS and/or TS). The markers rs109576799 and rs43696138, located in the *DRD3* and *HTR2A* genes, respectively, were significantly associated with both EV and TS traits. The CC genotype of the rs109576799 SNP had a significant effect on EV that was 1.06 and 1.03 m/s greater than the EVs of the heterozygous (CA, $p = 0.0025$) and homozygous (AA, $p = 0.0018$) genotypes; this locus was also significantly associated with TS. The CC genotype showed a TS that was 0.6 SD greater than those of the AA and CA genotypes ($p = 0.0045$ and $p = 0.0051$, respectively).

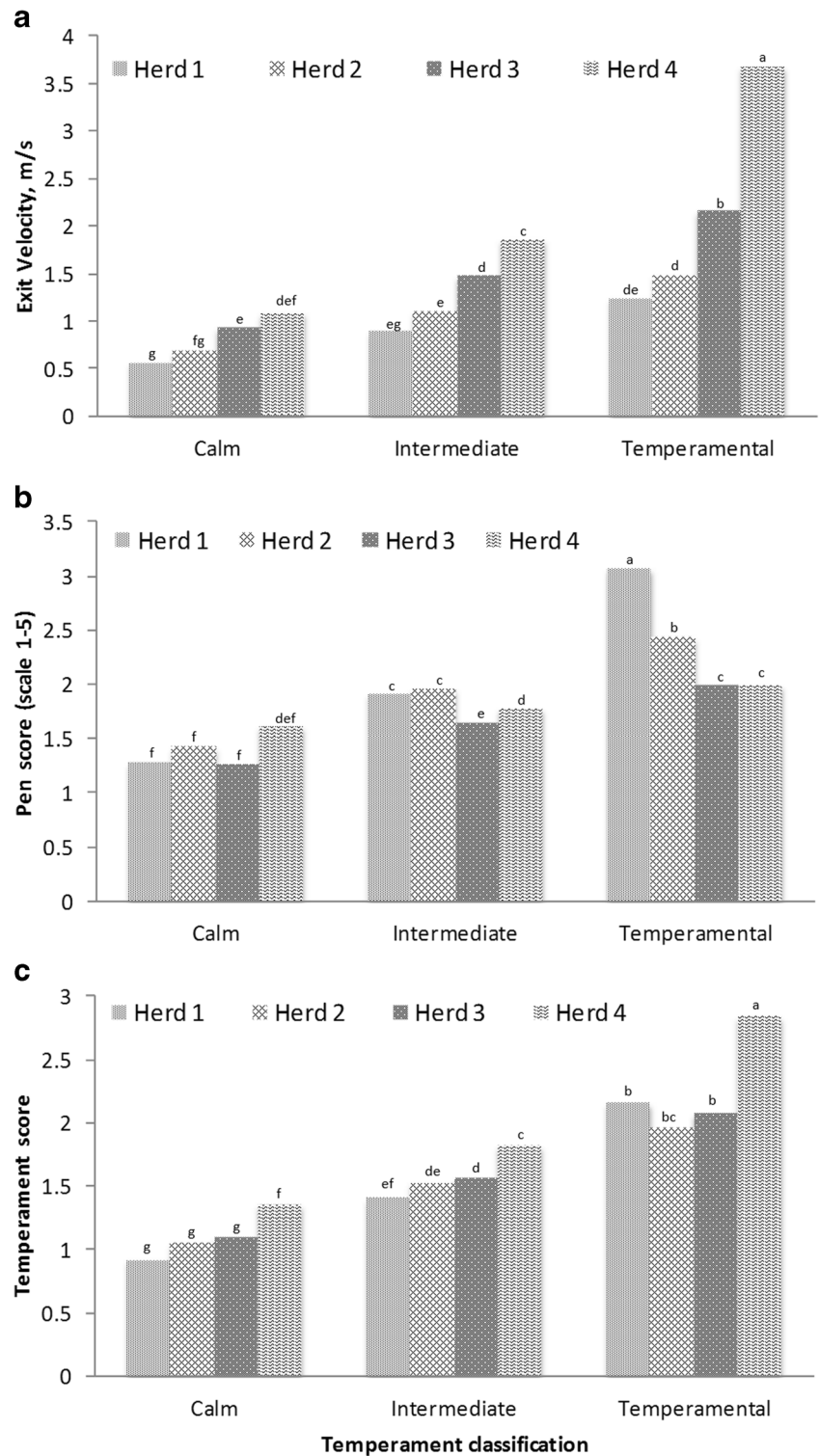
For the rs43696138 marker, the GA genotype had a significant effect on EV that was 0.09 and 1.21 m/s greater than the EVs of the homozygous (GG, $p = 0.0053$ and AA, $p = 0.06$) genotypes. TS was also affected by the genotype of this marker. The heterozygous GA genotype exhibited a TS that was 0.66 SD greater than those of the AA and GG genotypes ($p = 0.0393$ and $p = 0.0132$, respectively).

Four markers, rs110365063 and rs137756569 from the *POMC* gene and rs110365063 and rs135155082 located in *SLC18A2* and *DRD2*, respectively, were associated with PS; the most significant markers were rs110365063 and rs134604486. In the former, the GG genotype increased the PS value by 0.338 points compared with those of the AA and AG genotypes ($p = 0.05$ and $p = 0.01$, respectively); meanwhile, the PS of the homozygous TT genotype of marker rs134604486 was 0.28 and 0.53 points greater than those of the CT ($p = 0.06$) and CC ($p = 0.01$) genotypes, respectively.

Discussion

Animal behavioural genetic studies have been challenged by the definition of phenotypes (Mormède 2005). TS has been reported as a trait capable of accurately assigning beef cattle to calm, intermediate and temperamental classifications, and has been considered as a selection tool that combines two of the most commonly used temperament measures in cattle (i.e. EV

Fig. 1 Least squares means of exit velocity (a), pen score (b) and temperament score (c) by temperament groups in four herds of Charolais cattle. The different letters indicate significant differences ($p < 0.05$)

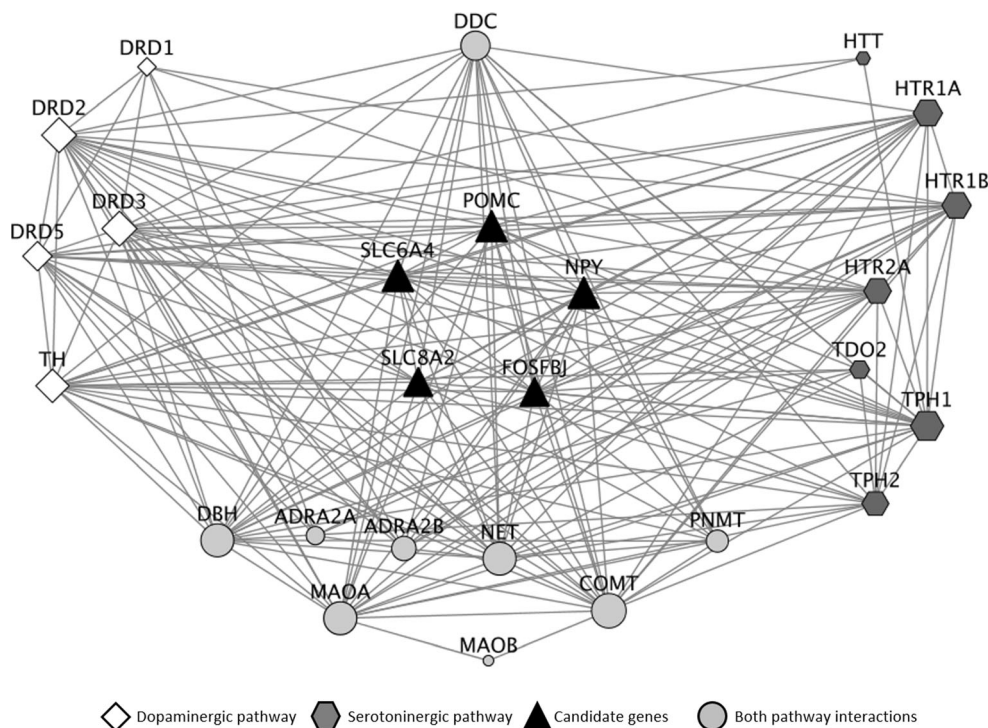


and PS) (Burdick et al. 2011); hence, we defined the groups of a Charolais cows’ population based on their TS to identify the genetic variants potentially associated with this behavioural trait.

We determined the TS based on the evaluation of EV and PS, which indicated that the Charolais population in our study

had temperament traits similar to previous reports in different cattle breeds, including *Bos taurus* and *Bos indicus* (i.e. Hereford, Angus, beef crosses Angus/Charolais and Brahman) (Nkrumah et al. 2007; Schmidt et al. 2014). As expected, the four herds studied had significant interactions with some environmental factors, with the most important

Fig. 2 Prioritisation of new genes as candidates for temperament in cattle. Five genes (black triangles) were found to be interacting with reference genes. The dot size of each represented gene indicates the strength of the interaction in the net



being age and handling management. The segregation of animals based on their TS allowed us to develop a study population that included the temperament-based extreme animals from the four herds.

Cattle temperament has been described as a complex trait. According to Cooke (2014), excitable temperaments have been positively associated with neuroendocrine stress reactions, regardless of the breed, age category and production system. Hence, genes related to the stress response, which involves the activation of a complex range of pathways, including the endocrine, nervous and immune systems, may potentially be associated with this trait (Smith and Vale 2006).

Due to their influence on almost all biological functions and the availability of their sequences in public databases, 24 genes that encode different regulatory enzymes,

transporters and receptors of the cattle serotonin and dopamine signalling pathways were considered as the obligate candidate genes for exploring the genetic variations associated with temperament. In addition, in view of the low numbers of genes and genetic variations known in cattle and in an effort to explore the genetic complexity of this trait, we used a PPI approach to increase the number of candidate genes. The use of the BosNet tool resulted in the prioritisation of five additional candidate genes: *POMC*, *NPY*, *SLC18A2*, *SLC6A4* and *FOSFBI*. Their potential to be associated with temperament was estimated based on their reported biological activities in humans and mice, which, with the exception of the *FOSFBI* gene, included interactions with neural activity, receptor function, targeting or synthesis of neurotransmitters and association with behaviour.

Table 2 Allelic frequencies, change type and *p*-values of associated single-nucleotide polymorphisms (SNPs) on temperament traits

Gene	SNP ID	Allele frequencies				Allele	Amino acid change	<i>p</i> -Values		
		A	C	G	T			PS	EV	TS
<i>DRD2</i>	rs135155082			0.926	0.073	G/T	V [Val] → V [Val]	0.0263		
<i>DRD3</i>	rs109576799	0.639	0.360			A/C			0.004	0.010
<i>HTR2A</i>	rs43696138	0.224		0.776		A/G	L [Leu] → L [Leu]		0.017	0.044
<i>POMC</i>	rs134604486		0.353		0.646	C/T		0.039		
	rs137756569	0.660		0.339		A/G		0.049		
<i>SLC18A2</i>	rs110365063	0.328		0.671		A/G	A [Ala] → T [Thr]	0.023		

PS Pen score, EV exit velocity, TS temperament score

POMC is the precursor for several peptide hormones produced by post-translational processing, some of which are involved in energy homeostasis, including α -melanocyte stimulating hormone (MSH), corticotrophic hormone (ACTH) and β -endorphin. In cattle, this gene plays an important role in ingestive behaviour, energy homeostasis and hot carcass and shipping weights (Zhang et al. 2009; Deobald and Buchanan 2011).

The *NPY* gene regulates appetite, feeding behaviour and hormonal activity. Because of its role in feeding behaviour, some SNPs of this gene have been associated with growth traits in cattle (Sherman et al. 2008).

Both *POMC* and *NPY* are key regulatory factors in the leptin/melanocortin pathway, which is considered one of the most important routes contributing to the regulation of energy metabolism; both are involved in the function of the hypothalamic–pituitary–adrenal axis, which plays an important role in the regulation of multiple physiological processes, including reproduction, anxiety, learning and memory, and cardiovascular function in both mammalian and non-mammalian species (Liu et al. 2014).

In humans, the vesicular monoamine transporter 2 (VMAT2, *SLC18A2*) is a critical mediator of dopamine dynamics in the neuronal terminal. VMAT2 is an H⁺-ATPase antiporter that uses the vesicular electrochemical gradient to drive the packaging of cytosolic monoamines (dopamine, serotonin, norepinephrine and histamine) into small synaptic and dense core vesicles (Lohr et al. 2015). Bovine VMAT2 was cloned and characterised by Krejci et al. (1993); these authors reported that the bovine adrenal medulla sequence, bVMAT, is more closely related to the transporters from the human and rat brains than to that from the rat adrenal medulla; however, there are no additional reports about their function or association with any trait in cattle.

The *SLC6A4* gene is located on chromosome 19 and functions as a serotonin transmembrane transporter and in serotonin binding. The serotonin transporter is a member of a highly homologous family of sodium-/chloride-dependent neurotransmitter transporters responsible for the reuptake of biogenic amines from the extracellular fluid. The serotonin transporter system is the pharmacological target of several clinically important antidepressants. Mortensen et al. (1999) reported the molecular cloning of the serotonin transporter from the bovine species, but no association studies have been reported in this species.

Finally, *FOSFBJ*, the human oncogene *c-fos*, is homologous to the Finkel–Biskis–Jinkins (FBJ) murine osteosarcoma virus oncogene. FOS was the first transcription factor identified that has a critical function in regulating the development of cells destined to form and maintain the skeleton. FOS is also a major component of the activator protein-1 (AP-1) transcription factor complex (Zhang et al. 1998). According to the functions reported, the *FOSFBJ* gene might be expected to

have a less specific effect on neural activity and behaviour than those genes targeting neurotransmitter synthesis and receptor function (*POMC*, *NPY*, *SLC6A4* and *SLC18A2*); however, it may be associated with the known phenotypic effects of different temperaments, i.e. loss/gain of weight and growth (Burrow and Dillon 1997; Cooke 2014).

As mentioned previously, molecular markers associated with behavioural traits in cattle are scarce; only two studies examined the association of polymorphisms located in candidate genes with different behavioural traits (Lühken et al. 2010; Glenske et al. 2011). Herein, we used a selective genotyping strategy to identify the molecular markers associated with temperament in Charolais cattle. Although this strategy has been considered suitable for QTL identification in cattle (Moioli et al. 2014), we justified the use of the temperament segregation for our association analysis with the multiple physiological studies that evaluated the effects of temperament on productive traits in cattle (Cooke 2014; Schmidt et al. 2014). Most of the cited studies used the segregation population analysis.

From a panel of 151 tested SNPs, we identified the associations of six SNPs from four genes with three temperament traits. It is important to remark that we based the study on the genetic diversity previously reported in databases for each gene; however, an additional advantage of using the segregated population is that it may be used to discover new genetic and Charolais-specific variants of these genes via resequencing of the proposed candidate genes.

We found two SNPs (rs109576799 and rs43696138) located in the *DRD3* and *HTR2A* genes, respectively, that were significantly associated with EV and TS. The biological functions of both genes have not been reported in cattle; however, in humans, genetic variants of the dopamine D3 receptor (*DRD3*) gene have been implicated in schizophrenia, autism and substance use disorders; the gene is also associated with emotional reactivity, executive function and stress response (Wolf et al. 2014). The bovine rs109576799 *DRD3* marker is located in an intron of the *DRD3* gene; while this variation had no obvious functional implication on gene expression that could explain its effect on temperamental traits, the predicted role of the gene in emotional reactivity and the sensitivity of the dopamine system to environmental stressors may potentially explain its association with behaviour.

The coding SNP rs43696138 in the bovine serotonin 5-hydroxytryptamine receptor 2a (*HTR2A*) gene is a synonymous mutation (Lys/Lys) and may be considered inconsequential for primary protein structure and/or function; however, a growing number of studies have shown that synonymous mutations are also under evolutionary pressure and can be implicated in complex traits and diseases, such as human cancer (Supek et al. 2014). In humans, the *HTR2A* gene is a G-protein-coupled excitatory receptor exerting its influence through the activation of the secondary messenger

phospholipase. Mutations in the human *HTR2A* gene are associated with susceptibility to schizophrenia and obsessive compulsive disorder, and, recently, polymorphisms of this gene have been proposed as a possible predisposition factor for aggressive traits (Banlaki et al. 2015).

Four additional SNPs associated with PS were located in three candidate genes, two (*SLC18A2* and *POMC*) of which were prioritised by the interactive network approach, and their functions were discussed above. The SNP rs110365063, located in bovine *SLC18A2*, is a transition that causes a change in the amino acid sequence from Ala to Thr. Its association with PS in cattle should be validated in further studies to explore its effects on the gene expression and/or protein function of *SLC18A2*.

The human D2 dopamine receptor is a G-protein-coupled receptor centrally involved in reward-mediating mesocorticolimbic pathways (Neville et al. 2004). Signalling through dopamine D2 receptors governs physiological functions related to locomotion, hormone production and drug abuse. D2 receptors are also known targets of antipsychotic drugs that are used to treat neuropsychiatric disorders such as schizophrenia. The dopamine receptor D2 is located on bovine chromosome 15 and has been described to play a role in regulating prolactin secretion. An SNP in this gene has been proposed as a marker to identify cattle that are resistant to the effects of endophyte-derived alkaloids (Campbell 2012). No studies have explored the role of this gene in cattle behaviour.

The extensive experimental evidence in humans and other non-human models supports our association results. Further attention must be paid to those polymorphisms showing important associations with temperament traits to validate their effects in other cattle populations.

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