Genome-wide Association Studies Identify Quantitative Trait Loci Affecting Cattle Temperament

Jiafei Shen
Northwest A&F University: Northwest Agriculture and Forestry University
Qiuming Chen
Northwest A&F University: Northwest Agriculture and Forestry University
Fengwei Zhang
Northwest A&F University: Northwest Agriculture and Forestry University
Quratulain Hanif
Pakistan Institute of Engineering and Applied Sciences
Bizhi Huang
Yunnan Academy of Grassland and Animal Science
Ningbo Chen
Northwest A&F University: Northwest Agriculture and Forestry University
Kaixing Qu
Yunnan Academy of Grassland and Animal Science
Jingxi Zhan
Yunnan Academy of Grassland and Animal Science
Hong Chen
Northwest A&F University: Northwest Agriculture and Forestry University
Yu Jiang
Northwest A&F University: Northwest Agriculture and Forestry University
Chuzhao Lei (leichuzhao1118@126.com)
Northwest Agriculture and Forestry University College of Animal Science and Technology
https://orcid.org/0000-0003-1647-1037

Research

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Abstract

Background

Cattle temperament is one of the most interesting traits owing to its correlation to productive efficiency, labor safety and animal welfare, but its genetic basis is not clearly understood.

Results

Here, we performed genome-wide association studies for a series of temperament traits, assessed in an open field and novel object test, using autosomal SNPs derived from the whole-genome sequence. We identified 37 and 29 genome-wide significant loci in an open field and novel object test, respectively. Gene set analysis implicated the neuroactive ligand-receptor interaction pathway. Analysis in tissue specific expressions showed enrichment in the brain. While some candidate genes were involved in psychiatric and neurodegenerative diseases in humans. The first principal component explained the largest variance in the data of open field and novel object test, and the most significant loci were assigned to *SORCS3* and *SESTD1*, respectively.

Conclusions

Our findings will facilitate cattle breeding for sound temperament by pyramiding favorable alleles to further improve the cattle production in the future.

Background

Animal temperament is usually used to describe a series of behavioral differences in response to the handling by humans, challenging situations, comprising various phenotypes including docility, aggressiveness, flight reactivity, emotion and response to novelty. Individual differences in animal temperament are attributed to genetics, age, sex, and environment (1). Temperament is also a major part of bovine behavior that influences animal welfare, production, health and fitness traits (2, 3). For example, the individual with poorer temperament exhibited lower average daily gain (4), worse reproductive efficiency (5), lower milk performance (6), inferior meat quality (7) and higher morbidity (8).

There are many measurement systems to detect animal temperament, e.g., open field test, novel object test, heart rate variability etc. Open field test (OFT) aims to test numerous behavioral characteristics of animals, like reactivity towards social isolation (9). In model animals, the test is usually used to investigate the physical response during emotional stress (10, 11). Novel object test (NOT) is used to assess the animal’s responsiveness to novel stimuli (12) and has been used to study learning and memory by behavior pharmacologists and neuroscientists (13). Heart rate variability (HRV) is a suitable approach for determining the activity of the autonomous nervous system in the study of temperament (14).

The temperament of cattle has been of greater interest since its domestication. Given different populations and metrical traits, there is a wide range of heritabilities, from low to high. For example, heritabilities for crush score and flight speed in Hereford cattle are 0.33 and 0.36, respectively [1], while heritability for flight speed in Nellore cattle is 0.21 (15). To date, many QTLs accounting for the variation on beef and dairy cattle temperament have been mapped, supporting a polygenic determinism. However, these studies were only based on SNP array or microsatellite markers to conduct the genome-wide association for a variety of temperament traits, such as aggressiveness at parturition (16), reactivity (3), flight speed (15), flight from feeder and social separation (17).

Recently, due to a continual decrease in genome sequencing cost, several studies use genome- wide autosomal variants to perform genome-wide association mapping. For example, a genome- wide association study (GWAS) utilized hundreds of canine whole genome sequences (WGS) to explore the relationship of 16 phenotypes including body weight with the genotype of the animal (18). In cattle, based on 25.4 million variants of the 1000 Bull Genomes Run4 reference population of 1,147 WGS individuals, a meta-analysis of GWAS revealed that 13.8% of the variance in stature was explained by 163 lead variants (19). However, scarce attempts have been made to identify candidate genes involving in cattle temperament using WGS data.

Brahman cattle is one of *Bos indicus* breeds which are relatively flighty compared with *Bos taurus* breeds (2, 20). Yunling cattle is a typical hybrid cattle breed in Brahman bull × Murray Grey bull/Yunnan indigenous cross cow, and thus it is an ideal model for studying the genetic basis of complex traits, such as temperament traits. In this study, we collected 15 phenotypic traits of open field test and 14 traits of novel object test in Brahman and Yunling cattle, and then separately performed principal component analysis for each test. Besides, we conducted a genome-wide association study of these 29 traits and the first principal component of open field test and novel object test using autosomal SNPs derived from WGS data. Our results provide new insights into the genetic basis of gentle temperament as well as credible information on genetic improvement of domestic cattle and for studying the mechanisms of behavioral abnormality in humans and animals.

Materials And Methods

Ethics statement

Ethics approval for all animal experiments was granted by the Institutional Animal Care and Use Committee of Northwest A&F University following the recommendation of the Regulations for the Administration of Affairs Concerning Experimental Animals of China.

Animal population

Individuals used for the detection of temperament traits comprised Brahman cattle and Yunling cattle. Yunling cattle were bred by Yunnan Academy of Grassland and Animal Science and all experimental animals were from its core breeding farm. All individuals were female, multiparous, and not at two weeks

pre-calving, calving and two weeks post-calving at the time of temperament assessment. In the feeding regime, the experimental animals comprised pen-feeding individuals and free-grazing individuals. The pen-feeding individuals were fed a total mixed ration composed of 65% grass silage and 35% concentrate on a dry matter basis. The free-grazing individuals ate grass in the pasture during summer and autumn (from June to November) every year and were properly fed above-mentioned TMR during winter and spring (from December to May) every year.

Temperament traits assessment

We combined open field test, novel object test and heart rate variability into a set of experiment procedures (Figure 1). Our assessment referenced to previous studies (14, 21) and performed for each animal in an open field area. 48 Brahman cattle (9 pen-feeding individuals and 39 free-grazing individuals) and 186 Yunling cattle (58 pen-feeding individuals and 128 free-grazing individuals) were selected in the behavioral experiments. Before the test, the selected individual was encouraged through a single file raceway to a squeeze crush to wear a heart monitor system (Polar V800, Polar Electro, Oy, Finland). Then, the animal walked along a single file raceway into the open field area for acclimatization within 10 mins, which was referred to as the period of open field test; subsequently, for the novel object test, a yellow duck doll was placed in the center of the area for 5 mins. The dimensions of the open field area and the duck doll were shown in S1 Figure. During the test, the animal's behavior was recorded by a video camera. Total time for contact with duck doll calculated by a stopwatch. The R-R data series was transformed into a computer and corrected with default parameters using gHRV (22). Eventually, the HRV data consisted of LHO, UFO, VFO, LFO, HFO, POO, MHO, HSO, PNO, RMO, APO, FDO, LHN, UFN, VFN, LFN, HFN, PON, MHN, HSN, PNN, RMN, APN and FDN. The other five traits consisted of STE, ACT, VOC, LAT and DUR. The full name, definition and significance of the 29 traits were shown in Figure 1 and S1 Table. Owing to some objective conditions, such as bad temperament, four Brahman cattle (two pen-feeding individuals and two free-grazing individuals) and 10 Yunling cattle (seven pen-feeding individuals and three free-grazing individuals) did not complete the novel object test.

Phenotypic analysis

We performed a general linear model to estimate the effect of breed on temperament traits with consideration of the effect of feeding regime using R project (23) To investigate the relationship among temperament traits, and between temperament traits and body measurement traits, we calculated the partial Pearson's correlation adjusting for feeding regime and breeds using the ppcor package (24) in R. Principal component analysis, conducted with princomp function of R project, was used to condense several correlated measures into a small number of principal components. The Kaiser-Meyer-Olkin (KMO) test of the measure of sampling adequacy was used to estimate the appropriateness of conducting PCA using REdaS package (25). The KMO test provided a value of 0.72 for the open field test and 0.66 for the novel object test. Because the correlation matrix with KMO > 0.6 is considered tolerable to explain the correlations between the variables (26), our data therefore appropriate for PCA analysis.

Sample collection and genome sequencing

After completing all behavioral assessments, we encouraged the test individual to the squeeze crush to collect ear tissue, whole blood and body measurement traits. Genomic DNA was extracted from the ear tissues using the standard phenol-chloroform protocol (27). A total of 158 paired-end libraries with an insert size of 350 bp were constructed and sequenced using Illumina NovaSeq (S2 Table). The length of the reads was 150 bp. The sequence data used in this paper was obtained from published papers where detailed information about sampling and sequencing was available (28). The aims of the whole blood and body measurement traits have been reported by previous study (29).

Reads mapping and SNP calling

Firstly, we mapped clean reads to the cattle reference assembly GCF_002263795.1 using BWA-MEM with default parameters (30). Duplicate reads were filtered using the "REMOVE_DUPLICATES=true" option of Picard tools. The average alignment rate and coverage were 99.54% and 5.61×, respectively. The "HaplotypeCaller", "GenotypeGVCFs" and "SelectVariants" argument of Genome analysis toolkit 3.8 (GATK) (31) were used for calling raw SNPs. The argument "VariantFiltration" of the same software was applied to all raw SNPs with following options: QD < 2, FS > 60, MQRankSum < -12.5, ReadPosRankSum < -8.0 and mean sequence depth (for all individuals) < 1/3× and > 3x. In addition, the haplotype-phase inference and missing alleles imputation were produced using Beagle (32) to carry out GWAS further. Based on ~41 M autosomal SNPs, we estimated the eigenvectors using smartPCA of EIGENSOFT v5.0 package (33) to adjust population structure in GWAS. The principal component 1 based on the genotype matrix could separate Brahman cattle from Yunling cattle (S2 Fig), which has been presented by a previous study (28).

GWAS analysis

A total of 13,006,271 SNPs and 12,948,424 SNPs were used in GWAS for 15 traits in open field test and 14 traits in novel object test, respectively. Meanwhile, the PC1 of each test was also acted as a phenotype for GWAS. For GWAS in the open field test, there were 30 Brahman genomes (5 pen-feeding individuals and 25 free-grazing individuals) and 128 Yunling genomes (29 pen-feeding individuals and 99 free-grazing individuals). For GWAS in the novel object test, there were 29 Brahman genomes (5 pen-feeding individuals and 24 free-grazing individuals) and 122 Yunling genomes (25 pen-feeding individuals and 97 free-grazing individuals). We carried out a GWAS with mixed linear model using genome-wide efficient mixed-model association (GEMMA) software package (34). For two-breed GWAS, the PC1 of autosomal SNPs, feeding regime and breed information were defined as the fixed effect. For within-Yunling-cattle GWAS, the PC1 of autosomal SNPs and feeding regime were defined as the fixed effect. The kinship matrix was defined as the random effect. Because the sample size of Brahman cattle is smaller, we did not carry out within-Brahman-cattle GWAS.

For multiple testing correction, because the effective number of independent SNPs for GWAS in open field test and novel object test calculated using PLINK (35) with the parameters (–indep-pairwise 50 5 0.2) were 750,367 and 737545, respectively, the P-value significant and suggestive thresholds were

approximately 5 × 10-8 (0.05/750,367) and 1 × 10-6 (1/750,367), respectively. In fact, the thresholds were widely used by numerous studies.

Proportion of variance explained (PVE) was defined as follows (36):

$$PVE = \frac{2\hat{\beta}^2 MAF(1-MAF)}{2\hat{\beta}^2 MAF(1-MAF) + \left(se(\hat{\beta})\right)^2 2NMAF(1-MAF)}$$

Where $\hat{\beta}$ is effect size for SNP maker, MAF is minor allele frequency for SNP marker, se$(\hat{\beta})$ is standard error of effect size for SNP marker and *N* is the sample size.

Functional annotation in the GWAS associated loci

To reveal important candidate genes, we used the following strategy to narrow down our findings. Firstly, we estimated the pairwise LD relation between associated SNPs. Borders of the associated loci were defined as the associated SNPs that are in approximate linkage equilibrium with each other at $r^2 > 0.6$ supplied by PLINK (35). To obtain important GWAS signals, the associated loci with the number of associated SNPs < 3 or the length < 1000 bp was excluded. Secondly, we also merged the loci in which the significant SNPs were associated with two or more temperament traits. In each independent locus, the SNP with the smallest *P*-value was called the leading SNP. Third, functional annotation of associated SNPs was carried out according to the *Bos taurus* reference genome (ARS-UCD1.2) in package ANNOVAR (37).

Pathway and QTL enrichment analysis

According to the functional annotation supplied by ANNOVAR, protein-coding genes located within 500 kb on either side of the associated SNPs were defined as candidate genes. For two-breed GWAS, there were 801 and 666 candidate genes for open field test and novel object test, respectively, whereas 89 candidate genes were overlapped. For each test, functional enrichment analysis was carried out on the list of candidate genes using the KEGG database supplied by KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/) (38). Moreover, we performed a chi-squared test using chisq.test function of R (39) to compare the observed and expected number of the independent loci overlapped with QTLs of each traits in Cattle QTL Database (https://www.animalgenome.org/cgi-bin/QTLdb/BT/index) (40).

Tissue expression analysis

A total of 85 RNA-seq experiments in *Bos taurus* were download from 5 studies of SRA database (PRJEB25677, PRJEB35127, PRJNA251439, PRJNA263600, PRJNA522422). All clean reads for each sample were aligned to the *Bos taurus* reference genome assembly using STAR (41) and hisat2 (42). Next, the transcript was assembly from alignment reads using Stringtie (43). Transcript FPKM was defined as the transcript expression levels using Ballgown (44). To identify the brain-specifically

expressed genes, we calculated tissue specificity indices (τ) as described earlier (45). Firstly, we filtered out weekly expressed genes in which the maximum expression level in all tissues was less than 1 FPKM. Second, τ is defined as follows:

$$\tau = \frac{\sum_{i=1}^{n}(1-\hat{X}_i)}{n-1};\ \hat{X}_i = \frac{X_i}{\max\limits_{1\le i\le n}(X_i)}$$

Third, we classified the genes as tissue-specifically expressed genes when τ > 0.8.

Results

Variation of temperament traits

To evaluate the difference in temperament, we measured 15 and 14 traits in an open field and novel object test, respectively, in 48 Brahman cattle and 186 Yunling cattle (Fig. 1) (S1 Table). The abbreviations and definitions of traits are shown in Fig. 1. In terms of feeding regime, Brahman cattle consisted of 9 pen-feeding individuals and 39 free-grazing individuals, and Yunling cattle consisted of 58 pen-feeding individuals and 128 free-grazing individuals. Diverse phenotypic variations of these traits are shown in the S3 Table for open field test and S4 Table for novel object test, respectively. It was observed that the coefficient of variation ranged from 2.89–248.26% for open field test and from 5.32–1300.90% for novel object test, respectively. The distributions of most temperament traits followed unimodal distribution, except for LAT (S3 Figure). We used a general linear model with consideration of the effect of feeding regime to test the effect of breed on temperament traits. Seven traits in Brahman cattle (FDO, APO, HSO, RMO, PNO, HSN and PNN) were significantly higher than those in Yunling cattle ($P < 0.05$) (Table 1), supporting previous observations that the docile *Bos taurus* was easier to handle than the relatively flighty *Bos indicus* (2, 20). Breed differences in temperament traits have also been reported in other studies (1, 14). These results suggested that the differences in temperament traits are, at least at the level of the breed, under some genetic control.

Table 1
Difference in temperament traits between in 48 Brahman cattle and 186 Yunling cattle (least square mean ± standard error).

Traits	Yunling	Brahman	P-value
HSO	31.7 ± 0.84	36.6 ± 1.67	0.00674
PNO	31.8 ± 1.61	39.1 ± 3.18	0.0337
APO	0.001 ± 0.0001	0.002 ± 0.0002	0.00587
FDO	1.018 ± 0.002	1.031 ± 0.004	0.00477
RMO	465 ± 28.0	598 ± 55.2	0.0264
HSN	30.5 ± 1.09	35.3 ± 2.12	0.0375
PNN	32.6 ± 1.77	43 ± 3.45	0.00561

To clarify the interrelationship of these 29 traits, we calculated partial correlation adjusting for feeding regime and breeds. A total of 256 pairs correlations became significant in 406 pairs of traits (0.143–0.988) (S4 Figure). In general, traits within each group mirrored one another and were tightly correlated. For example, PON was positively correlated with UFN (r_s = 0.949, $P = 5.14 \times 10^{-96}$), VFN (r_s = 0.967, $P = 1.33 \times 10^{-113}$), LFN ($r_s$ = 0.983, $P = 2.95 \times 10^{-142}$) and HFN ($r_s$ = 0.961, $P = 2.45 \times 10^{-107}$). In addition, we also evaluated the relationship between these traits and 15 body measurement traits (S4 Figure). A total of 75 pairs negative (0.144–0.435) and 5 pairs positive correlations (0.144–0.179) revealed significant in 435 pairs of body measurement traits and temperament trait. Among these correlations, the relationship between body length and LFO was the most significant correlation (r_s = -0.435, $P = 5.96 \times 10^{-10}$).

To condense the correlated temperament traits to fewer variables, we performed principal component analysis. The loadings of the temperament traits in the two principal components (PCs) gained from the PCA of the open field test was shown in S5 Table. Seven temperament traits (UFO, VFO, LFO, HFO, POO, RMO and APO) were found to load on the first principal component (PC1) with the highest factor loadings (> 0.3). The PC1 explained 50% of the variation in the data of open field test. Similarly, the loadings of the temperament traits in the two PCs gained from the PCA of a novel object test was shown in S6 Table. Six temperament traits (VFN, LFN, HFN, PON, RMN and APN) were found to load on PC1 with the highest factor loadings (> 0.3). The PC1 explained 51% of the variation in the data of the novel object test. These results suggested that the interpretation of PC1 was of importance to explore genetic mechanism underlying cattle temperament.

Two-breed genome-wide association studies for open field test

Based on ~ 13 million autosomal SNPs derived from the whole-genome sequence in 30 Brahman cattle and 128 Yunling cattle, we carried out a two-breed GWAS for 15 traits and PC1 in open field test, and

identified 173 significant SNPs and 974 suggestive SNPs (S5 Figure.) (S7 Table), which altogether implicated 801 candidate genes. Gene set enrichment analysis of Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways revealed that the most significant pathway was neuroactive ligand-receptor interaction (corrected $P = 2.42 \times 10^{-3}$) (S6A Figure.), which included many types of neuroreceptors genes, such as dopamine, serotonin, gamma-aminobutyric acid (GABA), and glutamate receptors. In fact, there were many neuroreceptors genes involved in cattle behavior, such as dopamine receptor D4 (45), glutamate ionotropic receptor AMPA 2 (46) and serotonin receptor 2A (47). Moreover, a previous study has demonstrated that genes interacting with anti-psychotic drugs for schizophrenia characterized by abnormality of emotional expression are overrepresented in the neuroactive ligand-receptor interaction pathway (48). Therefore, we inferred that the neuroactive ligand-receptor interaction pathway is critical to emotion control in cattle.

In order to provide more information about the functional impact of candidate genes on temperament, we utilized 85 RNA-seq experiments containing 18 main classes and 36 subclasses as described in Fig. 2A and 3D from SRA database in *Bos taurus* to identify brain-specifically expressed genes (S2 File) (Fig. 2A). The results showed 17,662 expressed protein-encoding genes, including 1646 brain-specifically expressed genes (FPKM ≥ 1). Amongst these brain-specifically expressed genes, 101 genes were found to be the candidate genes in open field test, more than expected by chance (χ^2 test, $P = 6.314 \times 10^{-3}$) (Fig. 2A).

We performed a series of linkage disequilibrium (LD) analyses for associated SNPs to count the number of LD blocks, leading to 37 significantly and 34 suggestively independent loci (S8 table). Among these independent loci, 29 loci were repeatedly observed in at least two traits. In addition, 12 out of 15 temperament traits had at least two associated loci, except for ACT, STE and MHO, which mainly supported a polygenic determinism (2).

In order to establish the relationship between these traits and other quantitative traits, we investigate the enrichment of QTLs derived from Cattle QTL Database in the independent loci found in open filed test. Of the 71 independent loci, 13, 14, 26 were overlapped with the QTLs of health, meat and carcass, and production traits, respectively, more than expected by chance (S9 Table). The results suggested that our temperament traits might be related with health, meat and carcass, and production traits.

In order to establish more contact between the independent loci and temperament, we surveyed the published literature about 33 genes closest to the significant loci. A total of 15 and 4 genes were found to be associated with psychiatric disease and neurodegenerative disease, respectively. For example, seven genes (*LOC539383*, *PPARA*, *NMUR2*, *TLR4*, *DAB2IP*, *PFKP*, *LOC788554*) were involved in schizophrenia (49–55). Other eight candidate genes were also involved in psychiatric disease in human, including autism spectrum disorder (*KLHDC7A*, *GABRG2, SLIT3, SHANK2*) (56–59), panic disorder (*MANEA*) (60), bipolar disorder (*KCNJ2*) (61), obsessive-compulsive disorder (*CDH2*) (62), and neuroticism (*SORCS3*) (63). Only four candidate genes were involved in neurodegenerative disease in humans, including multiple sclerosis (*CDK14*) (64) and Alzheimer's disease (*LZTS1*, *KHDRBS2*, *SORCS1*) (65–67). From the

above literatures, we could hypothesize that genes predisposing to psychiatric disease might play crucial roles in emotional control in domestic cattle.

For PC1, the most significant association locus was located in the first intron of *SORCS3* (Fig. 3A, 3B and 3C), a gene encoding sortilin related VPS10 domain-containing receptor 3. Moreover, we also found the *SORCS3* locus was associated with other five temperament traits (APO, HFO, LFO, POO and UFO) in open field test (S7 Table). In addition, the *SORCS3* region was the locus associated with the second largest number of traits in open field test after the *LZTS1* region. *SORCS3* is a neuronal receptor whose transcript is expressed at a prominent level in the cerebral cortex (68). In tissue expression profile, we also observed that the mRNA expression of *SORCS3* in the brain was higher than those in other tissues and *SORCS3* was a brain-specifically expressed gene ($\tau = 0.88$). (Fig. 3D). The previous study reported that the emotional-processing areas of the cerebral cortex could modulate the activity of the autonomic nervous system (69). Moreover, the experiment of *SORCS3*-deficient mice demonstrated that *SORCS3* is a postsynaptic modulator of synaptic depression and fear extinction (70). Besides, sortilin deficiency can prevent the age-dependent degeneration of sympathetic neurons (71). In our study, we combined an open field test and heart rate variability into an experimental procedure exploring the activity of the autonomic nervous system (sympathetic and parasympathetic) in emotional control. We thus concluded that this gene was a strong candidate gene contributing to emotional control in cattle.

Two-breed genome-wide association studies for novel object test

We also carried out a two-breed GWAS of 14 temperament traits and PC1 in the novel object test. We identified 154 significant SNPs and 847 suggestive SNPs (S5 Figure) (S10 Table), which altogether implicated 666 candidate genes. Gene set enrichment analysis of KEGG pathways revealed that the most significant pathway was axon guidance (corrected $P = 1.79 \times 10^{-4}$) (S6B Figure). Meantime, neuroactive ligand-receptor interaction (corrected $P = 0.016$) was also detected. Several studies have shown that the neuroactive ligand-receptor interaction pathway plays an important role in cognition-related diseases, such as Parkinson's disease (72) and Alzheimer's disease (73). Therefore, we inferred that neuroactive ligand-receptor interaction pathway is critical to cognitive function in cattle. We also found that 116 candidate genes were brain-specifically expressed genes, more than expected by chance (χ^2 test, $P = 1.450 \times 10^{-9}$) (Fig. 4B), suggesting that these candidate genes might work on central nervous system and further contribute to difference in temperament.

After calculating the linkage disequilibrium of associated SNPs to obtain independent loci, a total of 29 significant loci and 26 suggestive loci (S11 table) were associated with 13 temperament traits, and there were no associated loci for UFN. Among these independent loci, 11 loci were repeatedly observed in at least two traits. Moreover, 11 out of 13 temperament traits had at least two associated loci, except for HSN, MHN, suggesting that most of the temperament traits were quantitative traits controlled by polygenes. In addition, 14, 12, 22 of the 55 independent loci were overlapped with the QTLs of health,

meat and carcass, and production traits, respectively, more than expected by chance (S12 table), which indicates that our temperament traits might be related with health, meat and carcass, and production traits.

After surveying published literature about 20 genes closest to the significant loci, a total of six and four genes were found to be associated with neurodegenerative and psychiatric diseases, respectively. Among these genes, the most famous gene is *APP* (amyloid-β precursor protein), in which a mutation protected against Alzheimer's disease and age-related cognitive decline (74). In our study, *APP* locus was significantly associated with the total time at licking or sniffing the yellow duck doll, suggesting that *APP* was a strong candidate gene contributing to cattle cognition or recognition. The other five candidate genes were also involved in neurodegenerative disease in humans, including Alzheimer's disease (*NFATC2*, *CBLN4*, *CBFA2T3*) (75–77), cerebellar ataxia (*GRID2*) (78), and mental retardation (*RNF180*) (79). Only four candidate genes were involved in psychiatric disease, including bipolar disorder (*SESTD1*, *NR3C1*) (80, 81), obsessive-compulsive disorder (*SLITRK1*) (82), and mood disorder (*KCTD12*) (83) in humans. From the above literatures, we could hypothesize that genes predisposing to neurodegenerative disease might play crucial roles in cognition or recognition in domestic cattle.

For PC1, the most significant association locus was located in the first intron of *SESTD1* (Fig. 4A, 4B and 4C), a gene encoding SEC14 and spectrin domain containing 1. Moreover, we also found *SESTD1* locus to be associated with four other temperament traits (APN, HFN, FDN, RMN) in open field test. In addition, the *SESTD1* region was the locus associated with the largest number of traits in the novel object test (S9 table). The *SESTD1*, a developmentally dynamic synapse protein (84), is involved in lithium response (80). Interestingly, due to the neuroprotective effects of lithium, it has been regarded as a candidate drug in the disease-modification of neurodegenerative disorders, such as Alzheimer's disease and amyotrophic lateral sclerosis (85). Moreover, a previous study found that knockdown of *SESTD1* reduced dendritic spine density and excitatory synaptic transmission in hippocampal neurons (86). Numerous studies have demonstrated that the hippocampus plays a vital role in cognitive function (87, 88). Tissue expression analysis revealed that the mRNA expression of *SESTD1* in the brain was higher than those in duodenum, kidney, liver, spleen and muscle tissue (Fig. 4D). In our study, we combined the novel object test and heart rate variability into an experimental procedure detecting performance on cognition test. These results imply that *SESTD1* is a strong candidate gene contributing to cattle cognition or recognition.

Within-Yunling-cattle genome-wide association studies

Besides, we also carried out within-Yunling-cattle GWAS on temperament traits and PC1 in open field test and novel object test (S4 File). A total of 1648 suggestive SNPs and 2205 suggestive SNPs were associated with the temperament traits in open field test and novel object test, respectively. After detecting the linkage disequilibrium of these SNPs, a total of 110 suggestive loci and 152 suggestive loci were detected in open field test and novel object test, respectively. Among these association loci, 32 and 22 loci were overlapped in the regions detected in open field test and novel object test, respectively, through the two-breeds GWAS. It was to be observed that *SORCS3* locus and *SESTD1* locus identified by

two-breeds GWAS were also detected in the within-Yunling-cattle GWAS. Moreover, similar to the findings from a previous study (89), we also found the length of quantitative trait loci (QTL) identified by two-breed GWAS was shorter than that identified by within-Yunling-GWAS (t-test, $P = 0.03$ in open field test and $P = 0.08$ in novel object test), indicating that the two-breed approach provided smaller confidence intervals of QTL than within-breed analyses. This could explain by the utilization of the historical recombination events that have occurred in each breed, resulting in less linkage disequilibrium and better resolution (90).

Discussion

Along with the establishment of a correlation between temperament traits and economically important traits (e.g. production traits), cattle breeders lay more emphasis on docility in breeding programs in the future. In this study, open field test and novel object test were integrated into a set of experimental procedures. It is worth mentioning that we measured heart rate variability during the experimental procedure. Therefore, our experimental procedure could reflect the activity of the autonomous nervous system in emotional control and recognition of a novel object.

Based on temperament traits in open field and novel object test, we used ~ 13M SNPs from whole-genome sequence data to clarify the relationship between genotype and phenotype through two-breed GWAS, leading to 71 suggestive association loci (including 37 significant loci) in open field test and 55 suggestive association loci (including 29 significant loci) in novel object test, respectively. Although larger sample sizes are usually required for GWAS, the variants with more significant effects and high frequency could be identified using a smaller sample size. GWAS analysis with larger samples will identify additional variants with small effects and low frequency in the future.

Since WGS contain more variants compared with SNP array, our GWAS for temperament traits has a higher resolution and our candidate genes would be more plausible compared with previous studies. Moreover, although further functional experiments would allow us to validate our GWAS loci, most of the associated genes (e.g., neuroreceptors genes, brain-specifically expressed gene, psychiatric and neurodegenerative risk genes) have biologically plausible links to temperament traits. In addition, some strong candidate genes (e.g., *SORCS3*, *SESTD1* and *APP*) deserve more specific studies to confirm their putative role in modulating the emotional expression or cognitive processes.

Conclusions

In conclusion, in the present study, we collected a large number of temperament traits and associated genetic datasets. These results provide a theoretical basis for molecular-marker selection in the breeding and genetic manipulation of cattle temperament improvement to meet the increasing demand for better docility. Further explorations of causal genes underlying temperament will be necessary to perform genomic selection in domestic cattle and precision medicine in humans.

Abbreviations

LHO

low frequency/high frequency in open field test

UFO

ultra low frequency in open field test

VFO

very low frequency in open field test

LFO

low frequency in open field test

HFO

high frequency in open field test

POO

power in open field test

MHO

mean heart rate in open field test

HSO

standard deviation of heart rate in open field test

PNO

proportion of interval differences of successive intervals greater than 50 ms in open field test

RMO

root mean square of successive differences in open field test

APO

approximate entropy in open field test

FDO

fractal dimension in open field test

STE

the number of steps the animal moves in open field test

ACT

the time of activity in open field test

VOC

the number of vocalization in open field test

LHN

low frequency/high frequency in novel object test

UFN

ultra low frequency in novel object test

VFN

very low frequency in novel object test

LFN

low frequency in novel field test

HFN

high frequency in novel field test

PON

power in novel field test

MHN

mean heart rate in novel field test

HSN

standard deviation of heart rate in novel field test

PNN

proportion of interval differences of successive intervals greater than 50 ms in novel field test

RMN

root mean square of successive differences in novel field test

APN

approximate entropy in novel field test

FDN

fractal dimension in novel field test

LAT

time in s until lick or sniff the novel object was first shown

DUR

total time in s at licking or sniffing the novel object

Declarations

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Availability of data and materials

The raw whole genome sequencing data were reported in our previous study(28) and has been availabled at the NCBI Short Read Archive under the BioProject accession number PRJNA555741.

Author information

Affiliations

Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China

Jiafei Shen, Qiuming Chen, Fengwei Zhang, Ningbo Chen, Hong Chen, Yu Jiang & zhao Lei

Yunnan Academy of Grassland and Animal Science, Kunming, Yunnan 650212, China

Bizhi Huang, Kaixing Xu & Jingxi Zhan

National Institute for Biotechnology and Genetic Engineering, Pakistan Institute of Engineering and Applied Sciences, Faisalabad, 577, Pakistan

Quratulain Hanif

Contributions

Yu Jiang and Chuzhao Lei designed the study and supported the funding. Jiafei Shen and Qiuming Chen curated and analyzed the data. Jiafei Shen wrote the original manuscript. Quratulain Hanif reviewed and edited the manuscript. Fengwei Zhang, Kaixing Qu and Jingxi Zhan organized sampling and conducted fieldwork. All authors commented on the manuscript and gave final approval for publication.

Corresponding author

Correspondence to Yu Jiang and Chuzhao Lei

Ethics declarations

Ethics approval and consent to participate

This study was approved by Institutional Animal Care and Use Committee of Northwest A&F University following the recommendation of the Regulations for the Administration of Affairs Concerning Experimental Animals of China.

Consent for publication

Not applicable.

Competing interests

The authors declare no potential conflict of interest for this study.

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Figures

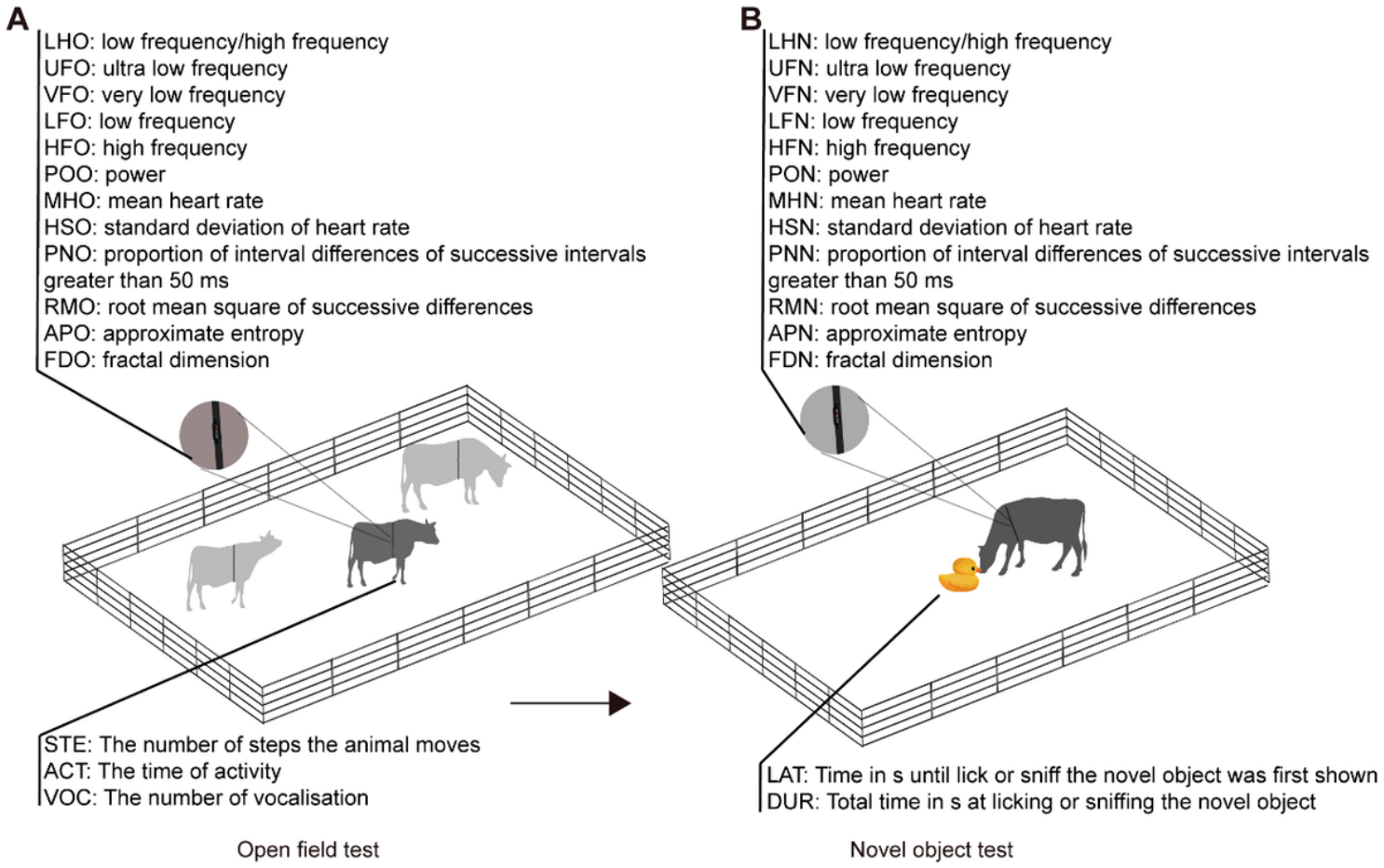
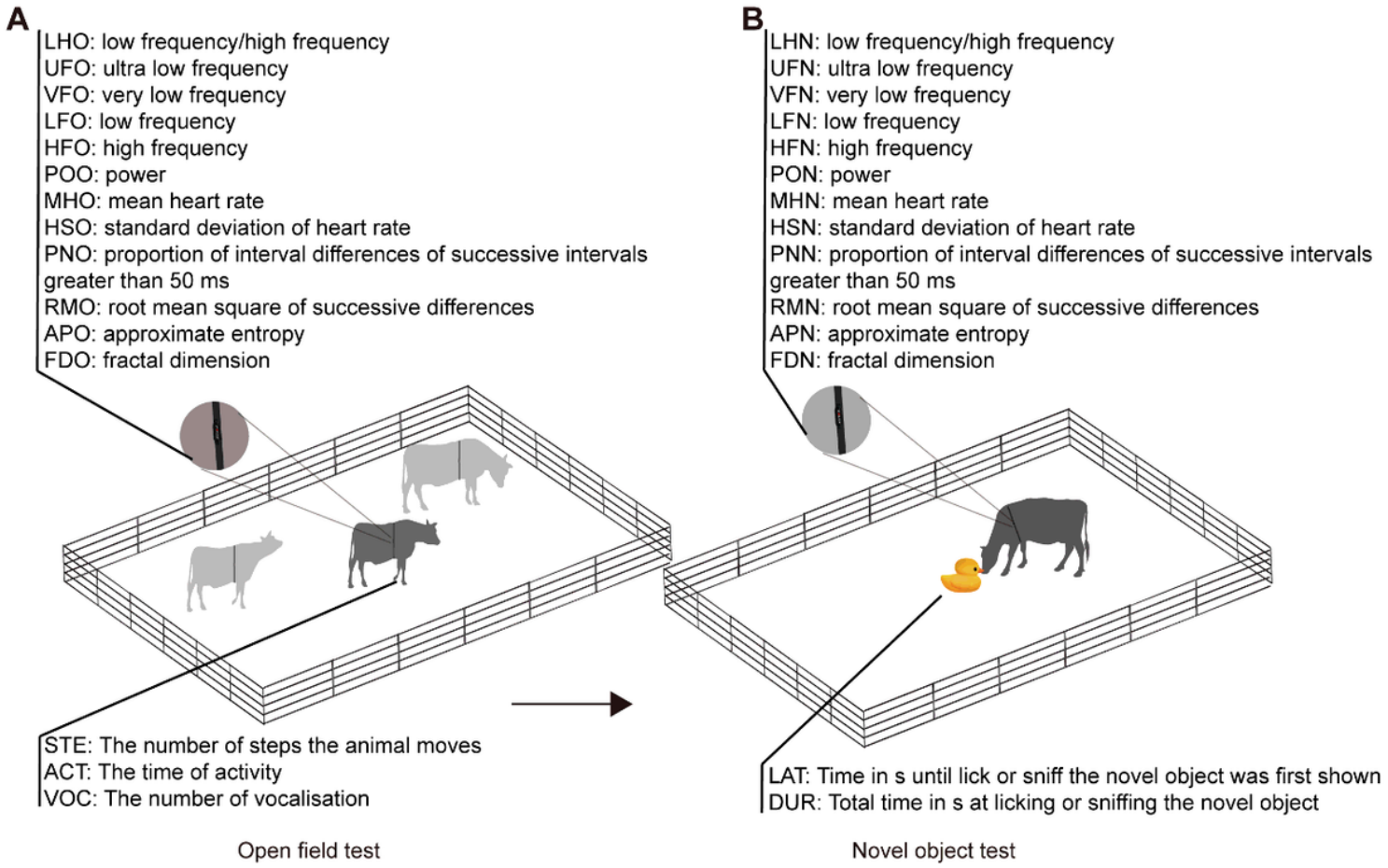


Figure 1

Schematic representation of open field test and novel object test. (A) Open field test with heart rate variation (HRV) data can be defined non-restrained test where the cow is free to remove within a defined testing area, and HRV test can measure the activity of autonomous nervous system. (B) Novel object test is used to assess the cow's response to a yellow duck doll. In our test, we can determine the activity of autonomous nervous system for different cognitive state. Compared with movement represented by gray, the movements represented by light gray happened earlier in schematics.



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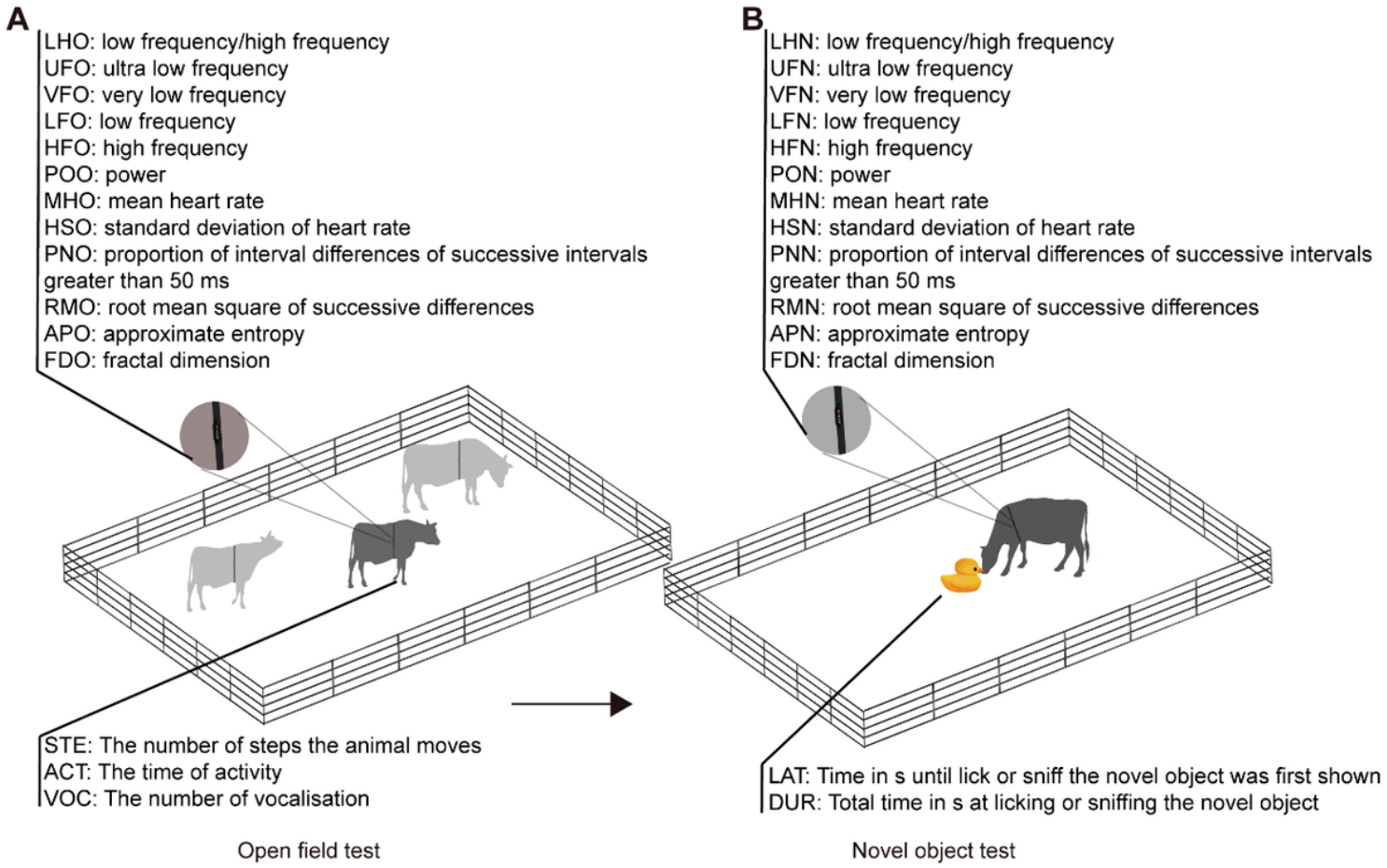

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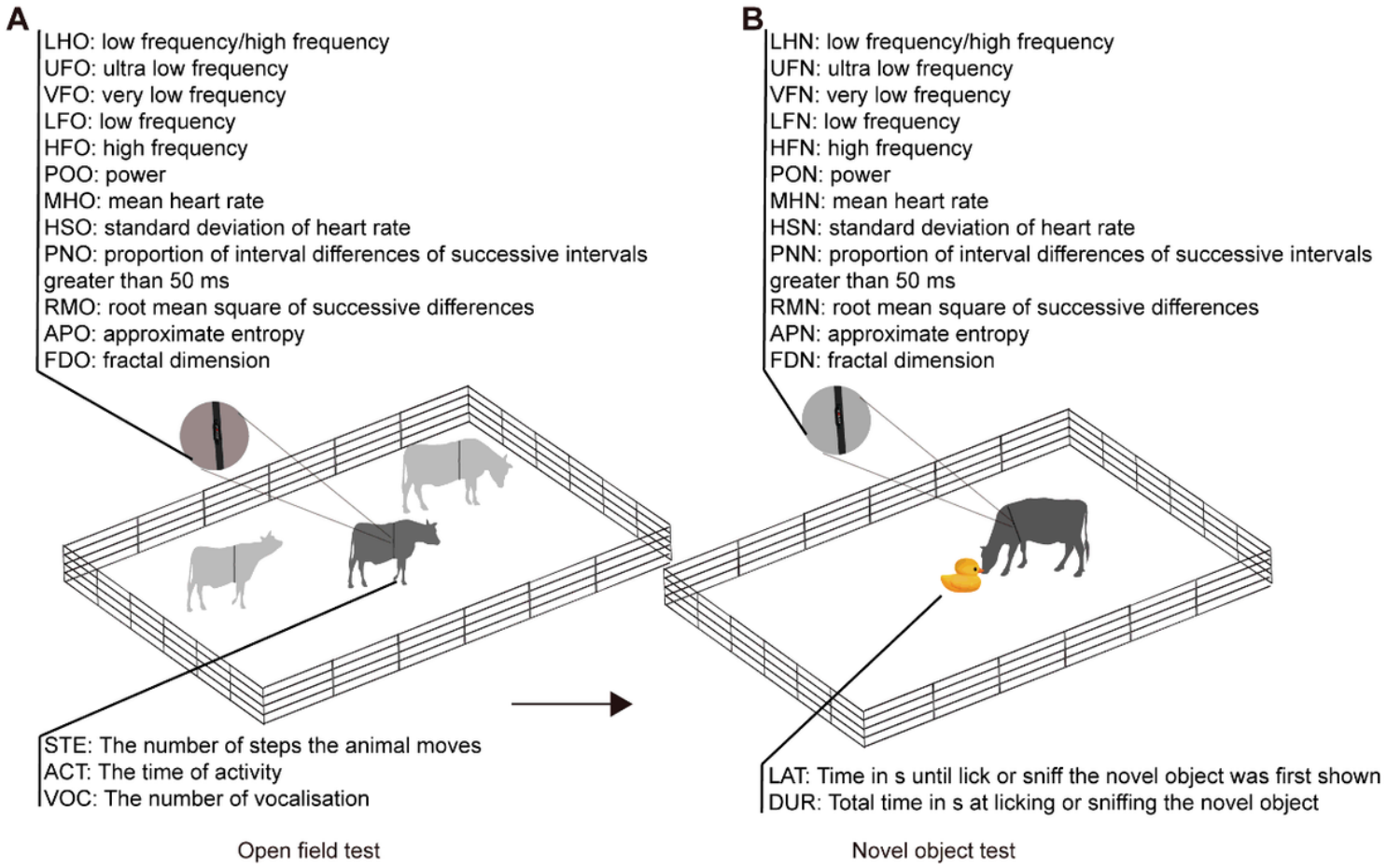

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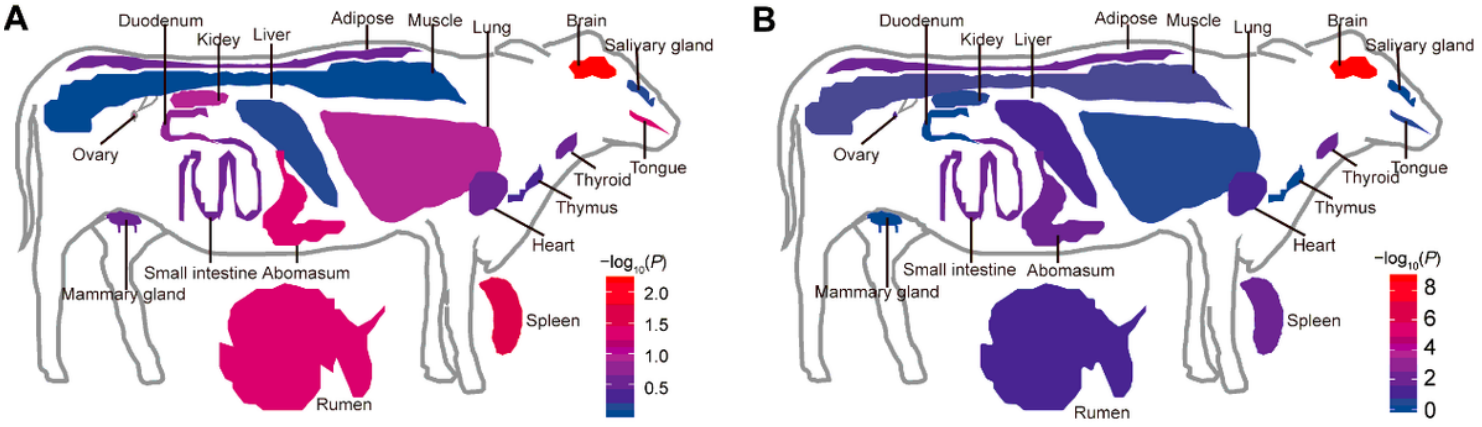
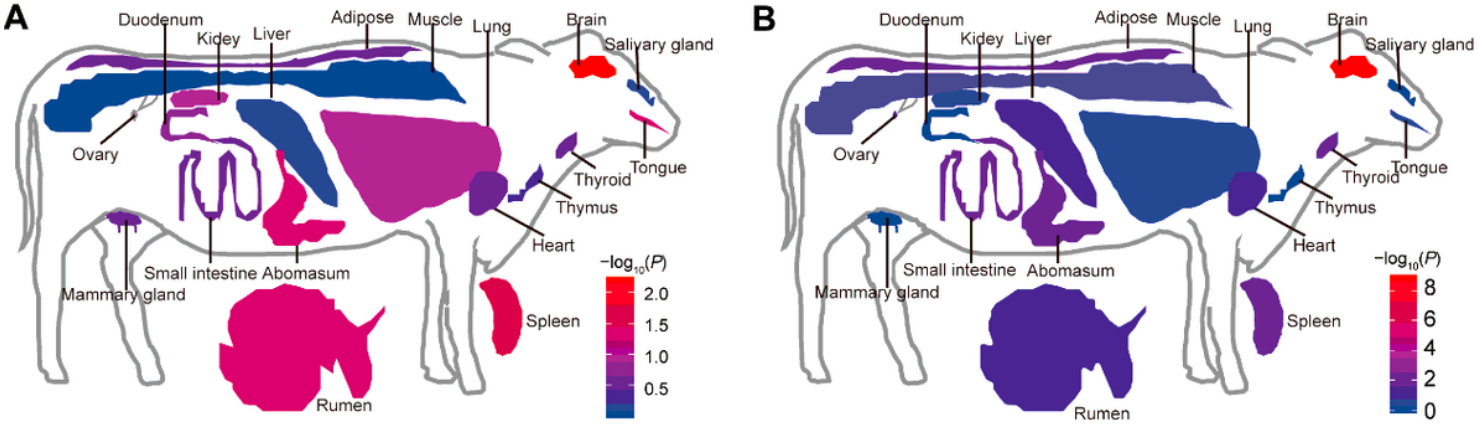


Figure 2

A x2 test comparing the observed and expected number of genes in each tissue to determine the predominant expression of candidate genes. (A) open field test. (B) novel object test.



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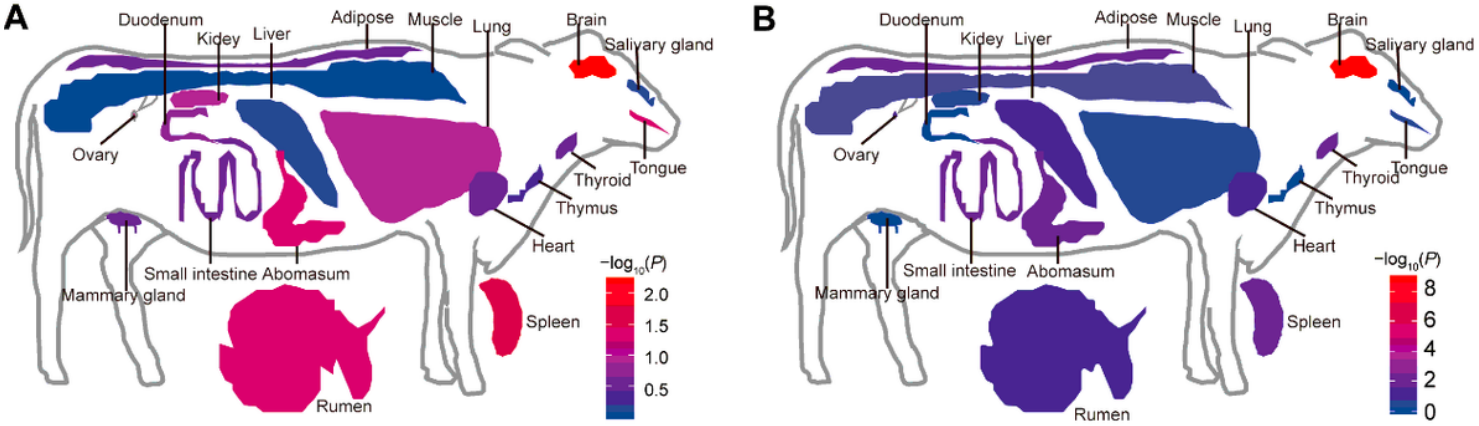

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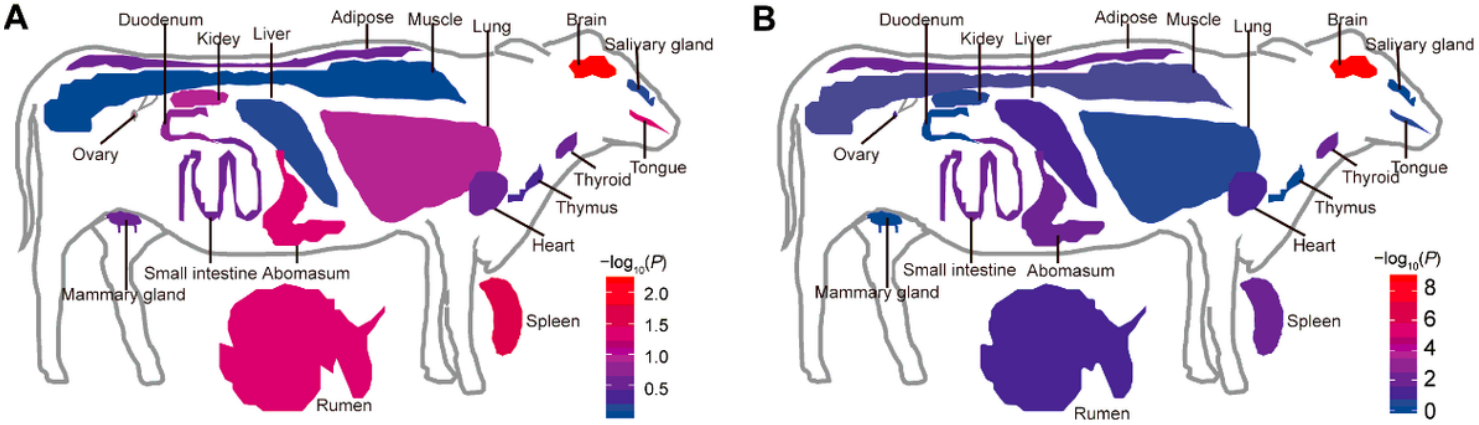

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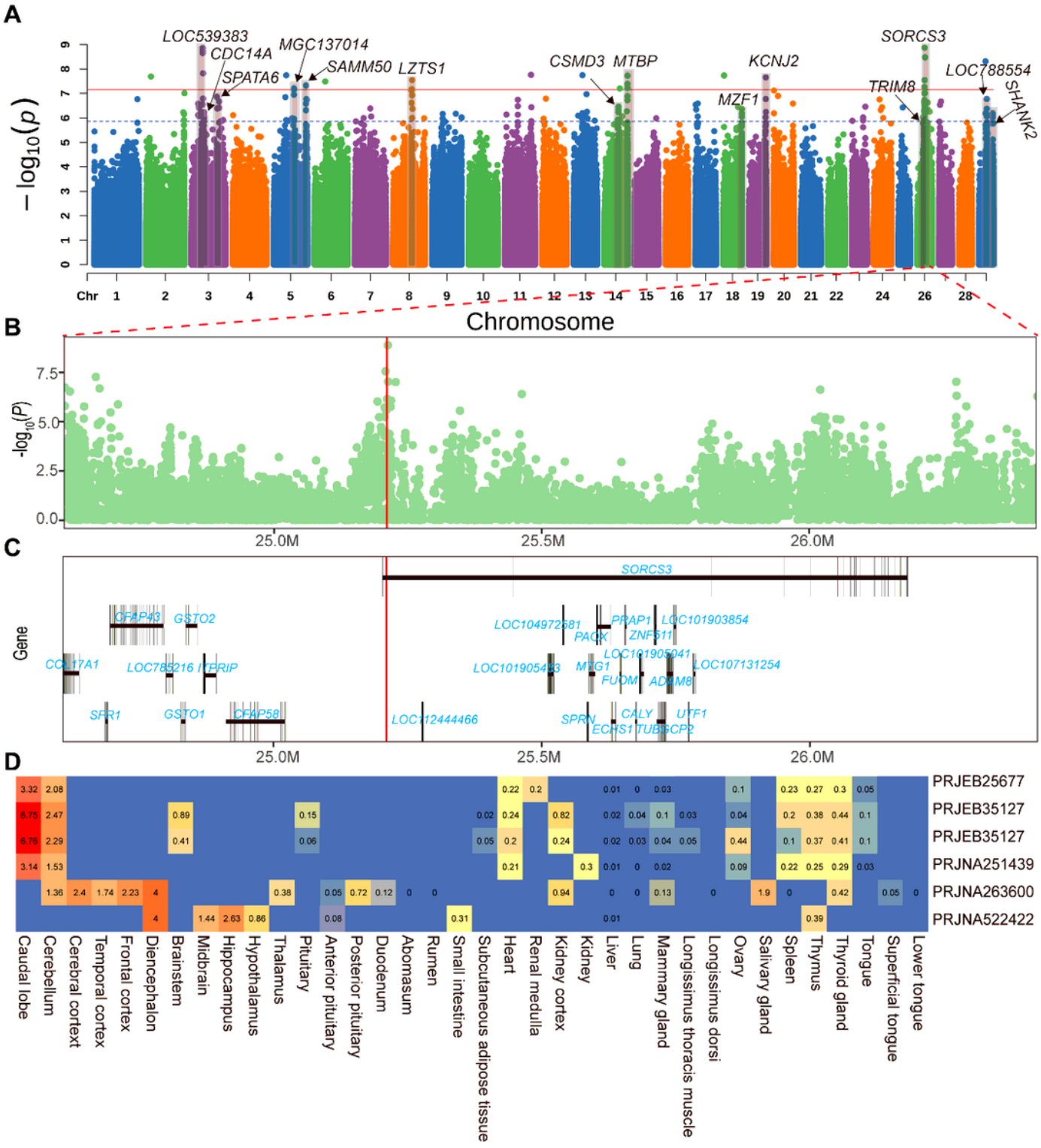
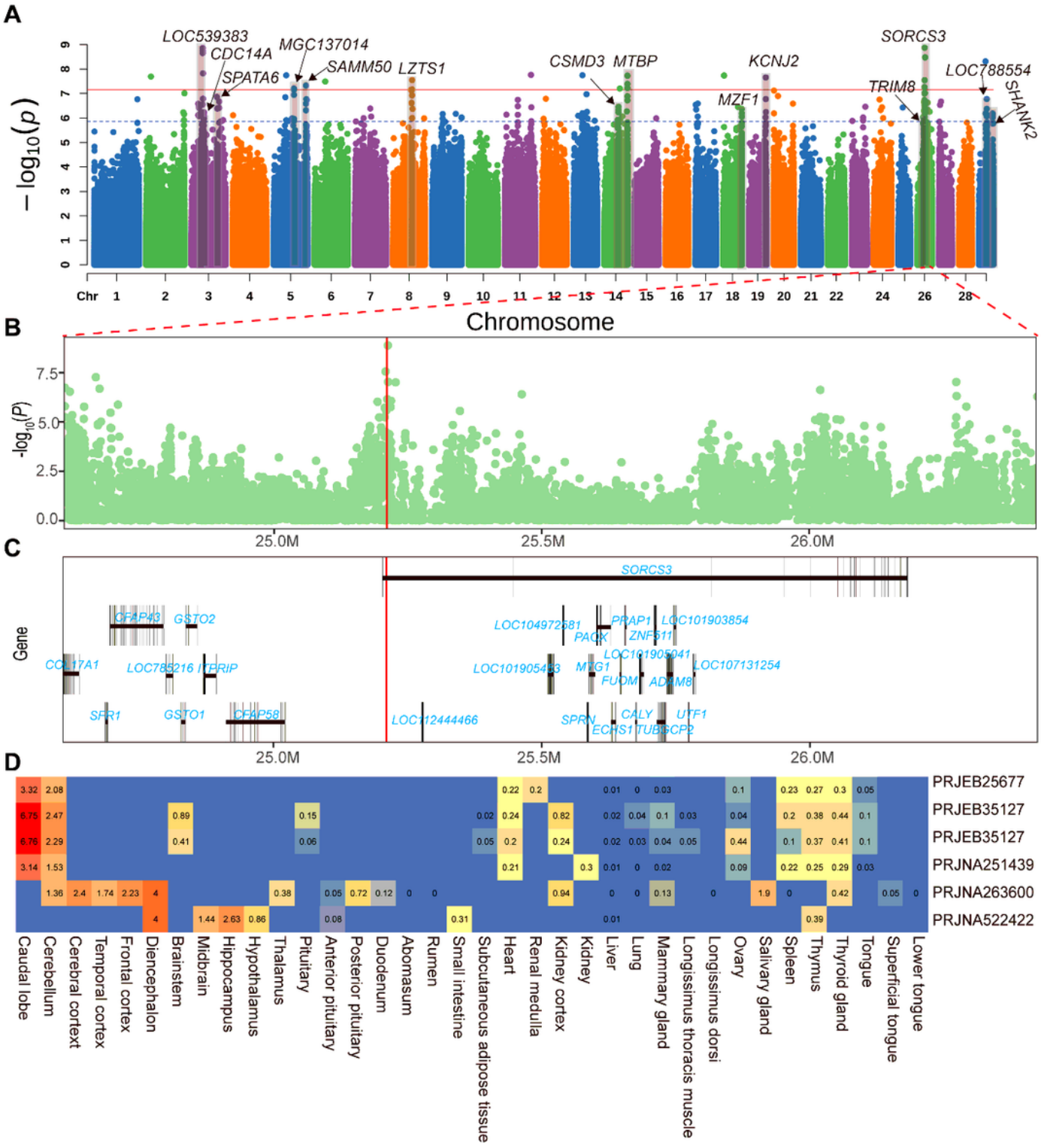


Figure 3

Identification of the strong candidate gene SORCS3 in the open field test. (A) Two-breed genome-wide association study of the first principal component in the open field test. The red line and blue line indicate the significant threshold and suggestive threshold, respectively. (B) Local Manhattan plot surrounding the

peak on chromosome 26. (C) Gene annotation of SORCS3 locus. Black rectangles and black line represent exons and introns, respectively. Red vertical line represents the position of leading SNP. (D) Gene expression of SORCS3 in different cattle tissues is based on 85 RNA-seq experiments from SRA database. The FPKM value is marked in the shade. Note that entries without FPKM value represent no data in the experiment.



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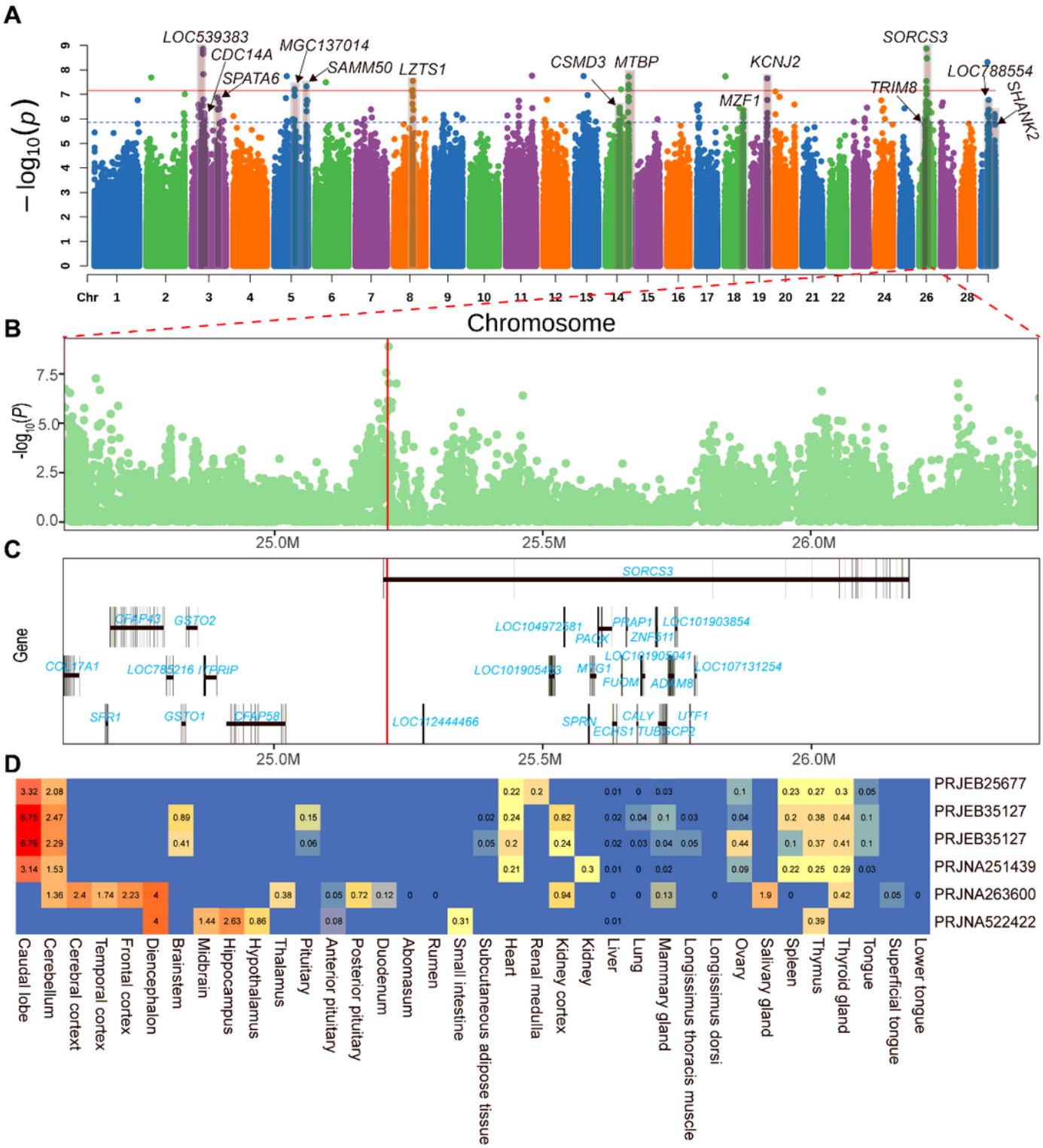

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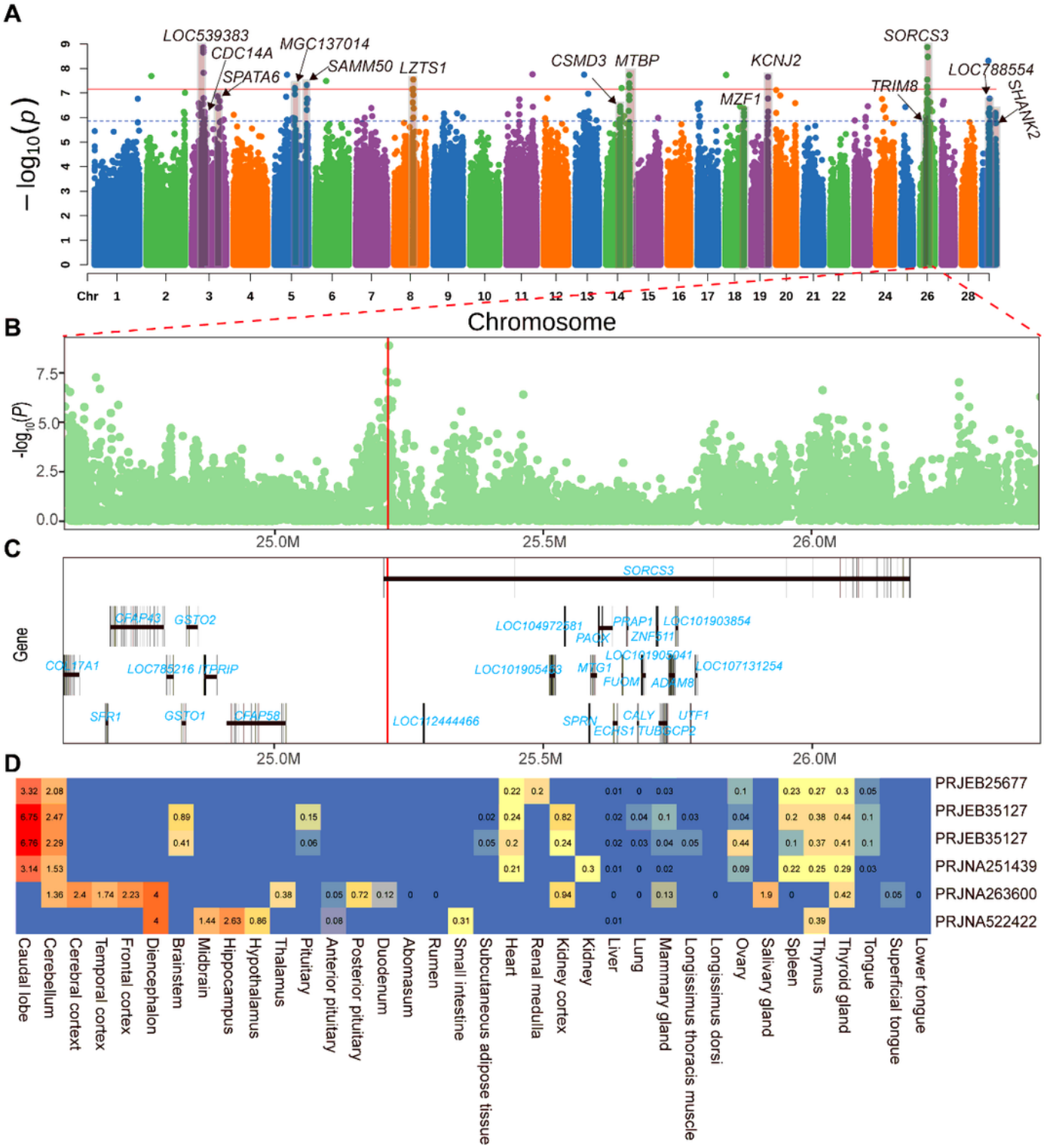

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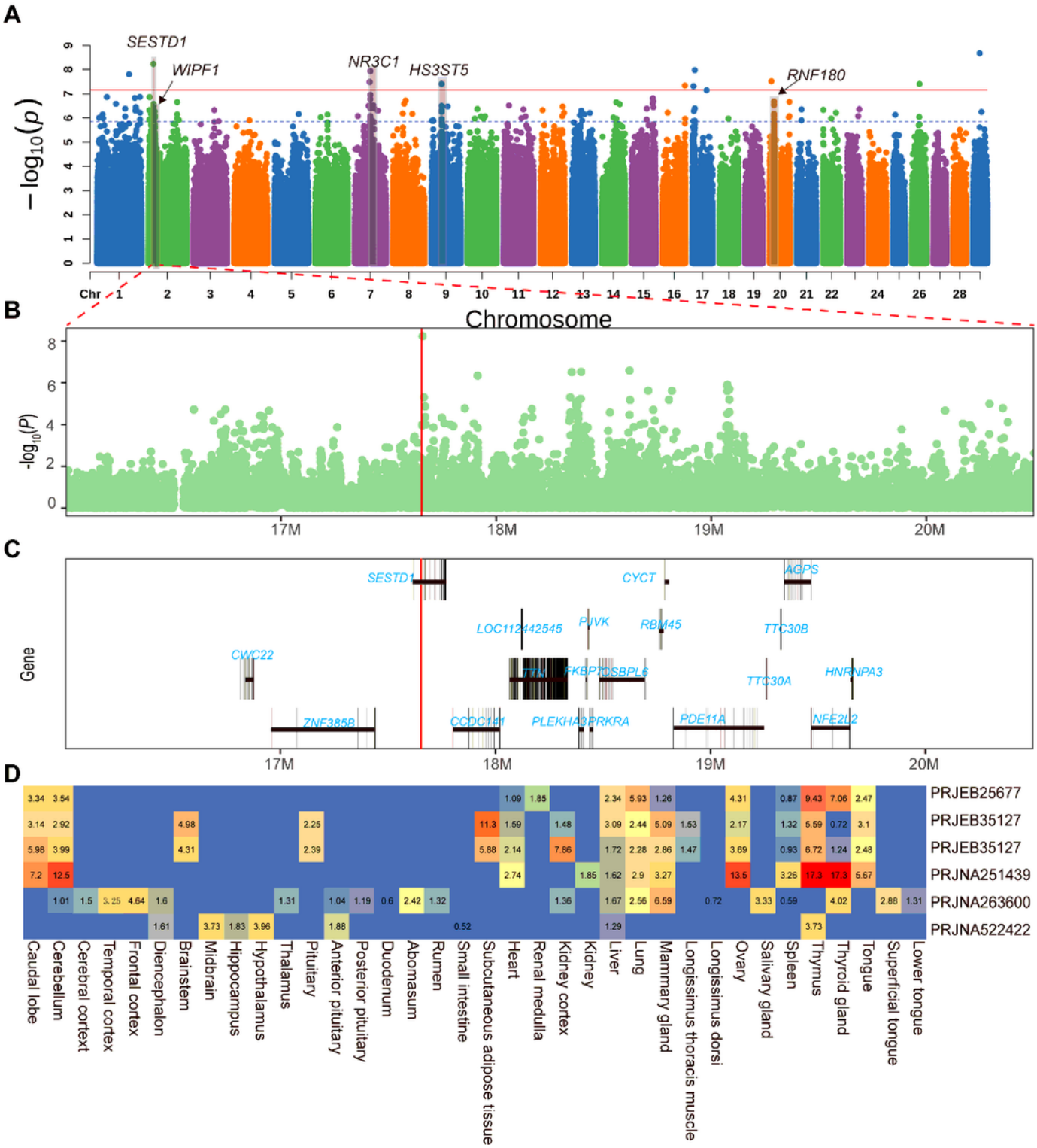
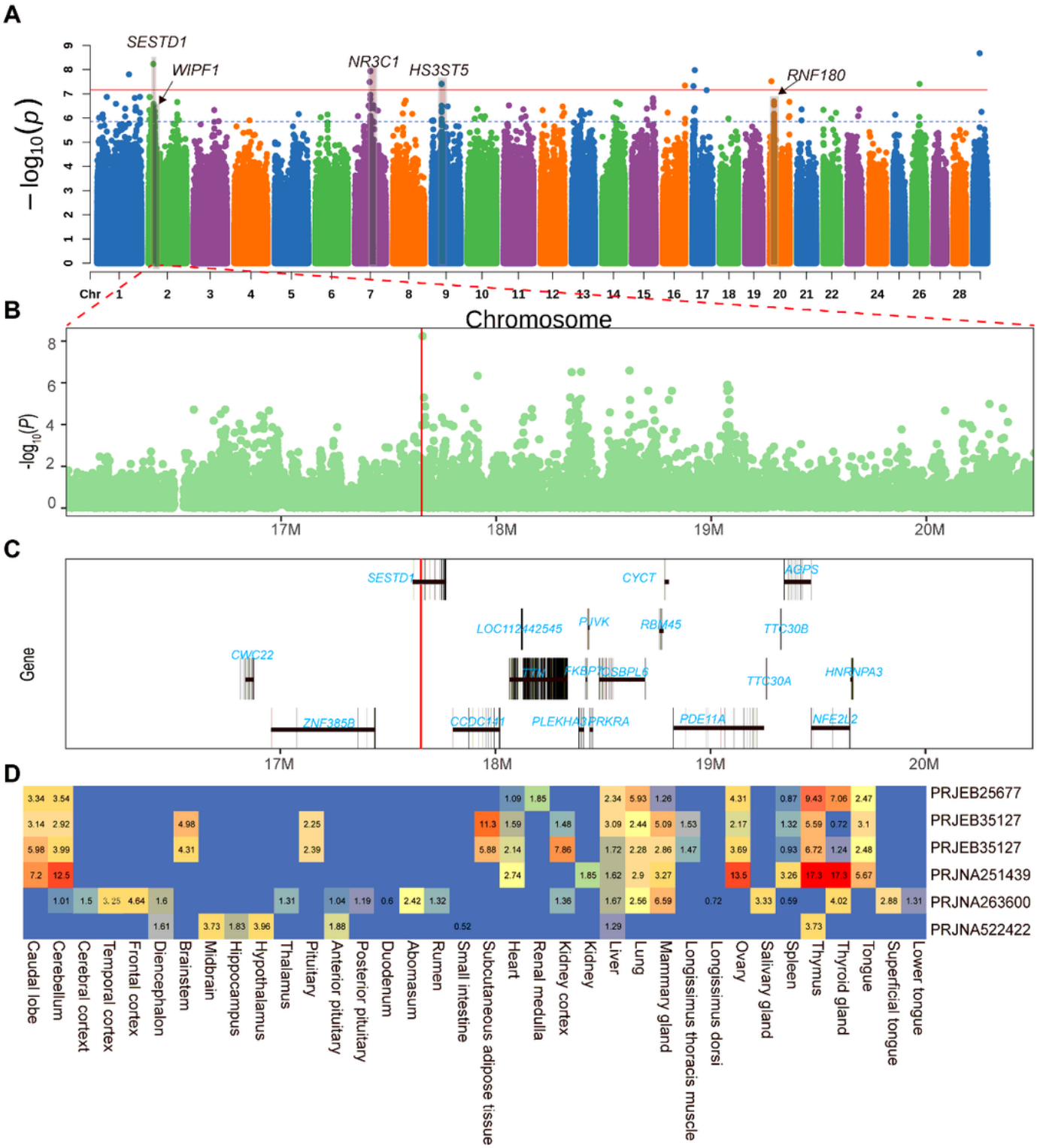


Figure 4

Identification of the strong candidate gene SESTD1 in the novel object test. (A) Two-breed genome-wide association study of the first principal component in the novel object test. The red line and blue line indicate the significant threshold and suggestive threshold, respectively. (B) Local Manhattan plot surrounding the peak on chromosome 2. (C) Gene annotation of SESTD1 locus. Black rectangles and black line represent exons and introns, respectively. Red vertical line represents the position of leading SNP. (D) Gene expression of SESTD1 in different cattle tissues is based on 85 RNA-seq experiments from SRA database. The FPKM value is marked in the shade. Note that entries without FPKM value represent no data in the experiment.



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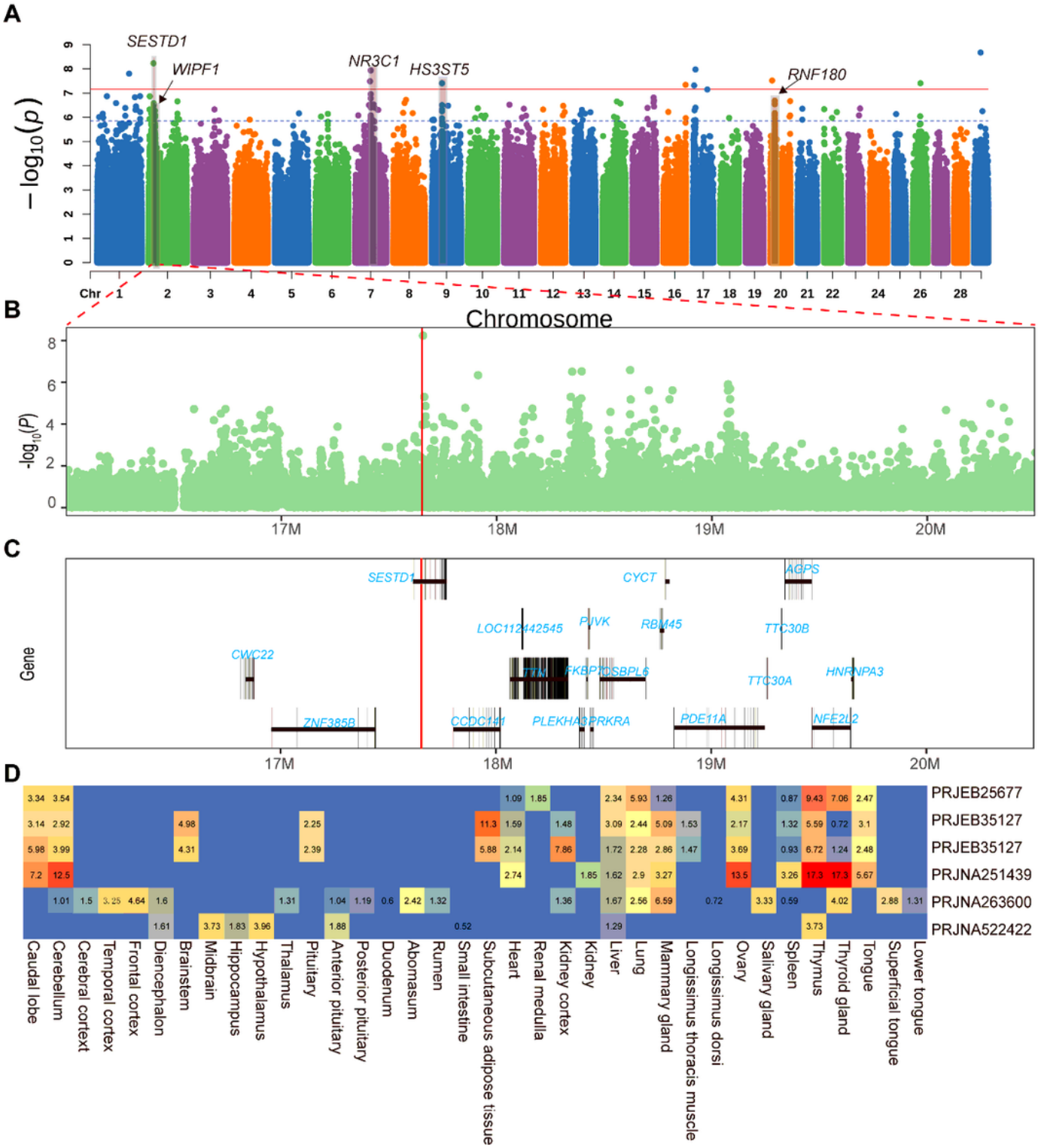

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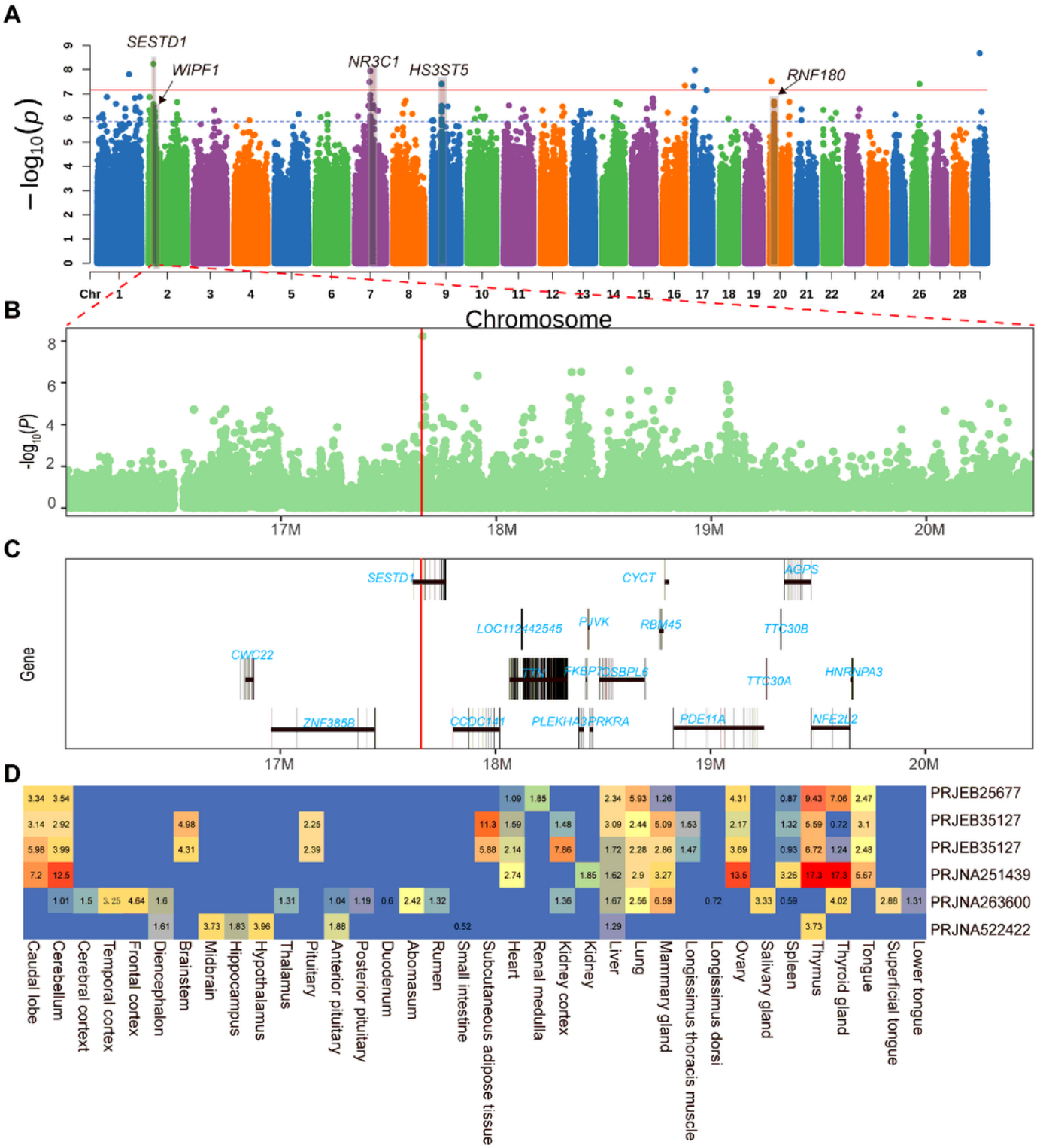

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