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检索报告

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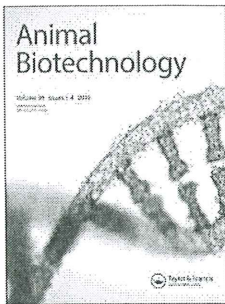
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A missense mutation in the bovine *NPBWR2* gene introgressed from *Bos javanicus* into Chinese indicine cattle

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ABSTRACT

Neuropeptide B/W receptor-2 (*NPBWR2*) is a G protein-coupled receptor which is related to the regulation of feeding behavior, energy homeostasis, neuroendocrine function, and inflammatory pain. In addition, it plays a potential role in the regulation of stress, emotion, anxiety and fear responses. Interestingly, previous genomic analyses revealed variable levels of *Bos javanicus* introgression into Chinese indicine cattle, including a key adaptive region encompassing the *NPBWR2* gene. By integrating introgression analysis with the Bovine Genome Variation Database, a banteng introgressed missense mutation (NC_083880.1: c.14C>G,Thr5Ser) of *NPBWR2* was identified. The current study explored the allele frequency of this mutation in 1648 individuals representing 63 Chinese cattle breeds/populations using polymerase chain reaction amplification and whole genome resequencing methods. Here, we explored the prevalence of this variant in native Chinese cattle to instigate the introgression influence of *B. javanicus* on Chinese cattle.

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1. Introduction

The modern domestic cattle were domesticated in two independent events in Southwest Asia ~10,000 years ago and in South Asia ~8,000 years ago, which are broadly classified into two major subspecies: the humpless taurine (*Bos taurus*) and the humped indicine/zebu (*B. indicus*).¹ Among modern cattle, at least eight major autosomal ancestral groups have been identified as follows: (1) African taurine cattle; (2) East Asian taurine cattle; (3) Eurasian taurine cattle; (4) European taurine cattle; (5) South Asian indicine cattle; (6) African indicine cattle; (7) Indonesian breeds; and (8) Chinese indicine cattle. These groups harbor various alleles from other wild and/or domestic Asian bovine species^{2,11}. In China, there are mainly three types of autosomal ancestral groups (East Asian taurine cattle, Eurasian taurine cattle, and East Asian indicine) with 58 recognized native cattle breeds^{9,10}, which are also influenced by aurochs (*B. primigenus*), yak (*B. grunniens*), gayal (*B. frontalis*), and banteng (*B. javanicus*).^{4,5,6,7,8}

Previously, genomic analysis revealed introgression events in Chinese indicine cattle, with 1,267 genes of banteng origin.¹² We have also discovered introgression events from banteng cattle to Chinese cattle, such as missense mutations in the *TAS2R16* bitter taste receptor gene.³³ Analysis across breeds reveals a clinal distribution of these variants, increasing in frequency toward southern China.³³ This pattern suggests they likely contributed to dietary adaptation in hot, humid environments.³⁰ The average banteng ancestry proportion was found to be 1.13–10.21%, and the highest proportion of banteng ancestry was observed in cattle from the

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southeastern coastal region of China.¹³ The introgressed regions from *B. javanicus* has enhanced the genomic diversity of Chinese indicine cattle and contributed to their rapid adaptation to tropical and subtropical environments, such as *NPBWR2*,¹⁴ which has been implicated in the regulation of feeding behavior, energy homeostasis,¹⁶ and neuroendocrine function and inflammatory pain modulation.¹⁷ *NPBWR2* gene encodes an evolutionarily conserved G protein-coupled receptor implicated in neuroendocrine and immune regulation.¹⁵ Throughout their adaptation to diverse agroecological environments, cattle populations have undergone genomic introgression and selective sweeps affecting loci involved in immunity, thermotolerance, and energy metabolism. *NPBWR2* may contribute directly to these adaptive processes.⁸

Using the Bovine Genome Variation Database (BGVD),¹³ a missense mutation in *NPBWR2* (NC_083880.1: c.14C>G) was identified, which was specified to Chinese indicine cattle and originated from *B. javanicus*. In view of the above physiological parameters, variations within this gene might influence the behavioral and metabolic adaptation that render Chinese cattle to better tolerate the nutritional and thermal challenges across tropical and subtropical regions. Therefore, based on the previous findings, the current study aims to further characterize the important functional mutations of the *NPBWR2* gene in 63 Chinese indigenous cattle breeds/populations. We investigated its polymorphism and distribution in Chinese indicine cattle to provide a theoretical basis for subsequent related research and breed improvement programs.³

2. Materials and methods

2.1. Sample collection

The current study was carried out in strict accordance with national and institutional guidelines for animal welfare. The experimental protocol was approved by the Faculty Animal Policy and Welfare Committee of Northwest A&F University (FAPWC-NWAFU; protocol number WAFAC1008). The study cohort comprised 1648 individuals from 63 Chinese indigenous breeds/populations (Table 1). Additionally, *NPBWR2* genotypes were extracted from publicly available whole genome sequencing data of Chinese cattle and 10 banteng individuals, applying previously described criteria.^{18,19,12}

2.2. Genomic DNA extraction and PCR amplification sequencing in Chinese indicine cattle

The genomic DNA was extracted from 941 ear tissues using the standard phenol chloroform method.²⁰ The polymerase chain reaction (PCR) primers were designed on the basis of the bovine *NPBWR2* sequence (NC_083880.1) on the *B. taurus* autosome (BTA) 13. A single pair of primers (forward: 5'-CCTTCCAGGTCACAAAACCGT-3' and reverse: 5'-AAGGTGGCATTGTGTCTGGT-3') was designed to amplify a 144bp product. Each 12.5 µL reaction contained 20 ng of genomic DNA, 20 pmol of each primer, 0.25 mM dNTPs, 1×PCR buffer (including 2.5 mM Mg²⁺), and 1.0 U of rTaq DNA polymerase (Takara, Dalian, China). The amplification program consisted of initial denaturation at 95°C for 10 min; 32 cycles of denaturation at 94°C for 30 s, annealing at 58.5°C for 30 s, and extension at 72°C for 30 s; followed by a final extension step at 72°C for 10 min. The PCR products were visualized by 1% agarose gel electrophoresis, while the samples exhibiting clear bands were subjected to Sanger sequencing.

2.3. Genotype of whole genome resequencing data

Whole genome resequencing data from 761 individuals was obtained from previous studies as well as from public databases. The SNPs within the *NPBWR2* genomic region were extracted and genotyped using a standardized variant-calling procedure.⁸ Genotypes were filtered based on read depth and quality scores. Subsequent allele and genotypic frequencies were calculated directly from these curated calls.

Table 1. Genotype and allele frequency of the *NPBWR2* gene c.14C>G locus in 63 Chinese indigenous breeds/populations and 2 populations from other regions.

Group	Breeds	Number	Genotype frequencies			Allele frequencies	
			CC	CG	GG	C	G
Northern group	Chaidamu(CDM)	13	0.6154(8)	0.3077(4)	0.0769(1)	0.7692	0.2308
	Dingjie(DJ)	32	0.9688(31)	0.0312(1)	0.0000(0)	0.9844	0.0156
	Shigatse Humped(SH)	24	1.0000(24)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Ganzi(GZ)	28	0.8929(25)	0.1071(3)	0.0000(0)	0.9464	0.0536
	Mongolian(MG)	21	1.0000(21)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Hetian(HT)	20	1.0000(20)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Yushu(YS)	24	1.0000(24)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Zhangmu(ZM)	3	0.6667(2)	0.3333(1)	0.0000(0)	0.8333	0.1667
	Linzhi(LZ)	18	1.0000(18)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Yanbian(YB)	18	1.0000(18)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Kazakh(KH)	2	1.0000(2)	0.0000(0)	0.0000(0)	1.000	0.0000
	Anxi(AX)	13	0.8462(11)	0.1538(2)	0.0000(0)	0.9231	0.0769
	Fuzhou(FZ)	21	1.0000(21)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Zaosheng(ZS)	20	0.3500(7)	0.4000(8)	0.2500(5)	0.5500	0.4500
Central group	Guyuan(GY)	60	0.4000(24)	0.4667(28)	0.1333(8)	0.6333	0.3667
	Jiaxian red(JX)	35	0.6000(21)	0.2571(9)	0.1429(5)	0.7286	0.2714
	Bohai(BH)	23	0.8261(19)	0.1304(3)	0.0435(1)	0.8913	0.1087
	Zaobei(ZB)	10	0.8000(8)	0.2000(2)	0.0000(0)	0.9000	0.1000
	Jinnan(JN)	20	0.9000(18)	0.1000(2)	0.0000(0)	0.9500	0.0500
	Qingchuan(QC)	20	0.8500(17)	0.1000(2)	0.0500(1)	0.9000	0.1000
	Luxi(LX)	66	0.6818(45)	0.3030(20)	0.0152(1)	0.8333	0.1667
	Mengshan(MS)	36	0.6944(25)	0.2778(10)	0.0278(1)	0.8333	0.1667
	Nanyang(NY)	16	0.6875(11)	0.2500(4)	0.0625(1)	0.8125	0.1875
	Bashan(BS)	5	0.2000(1)	0.6000(3)	0.2000(1)	0.5000	0.5000
	Yiling(YL)	48	0.5417(26)	0.1667(8)	0.2917(14)	0.6250	0.3750
	Lincang(LC)	22	0.9091(20)	0.0000(0)	0.0909(2)	0.9091	0.0909
	Dabieshan(DBS)	33	0.5152(17)	0.3333(11)	0.1515(5)	0.6818	0.3182
	Xianan(XN)	34	0.9412(32)	0.0588(2)	0.0000(0)	0.9706	0.0294
Southern group	Minnan(MN)	11	0.5455(6)	0.3636(4)	0.0909 (1)	0.7273	0.2727
	Chaling(CL)	20	0.3500(7)	0.6000(12)	0.0500(1)	0.6500	0.3500
	Nandan(ND)	39	0.6667(26)	0.2821(11)	0.0513(2)	0.8077	0.1923
	Wannan(WN)	30	0.6333(19)	0.0000(0)	0.3667(11)	0.6333	0.3667
	Weizhou(WZ)	36	0.5833(21)	0.3611(13)	0.0556(2)	0.7639	0.2361
	Wandong(WD)	2	0.5000(1)	0.5000(1)	0.0000(0)	0.7500	0.2500
	Wenshan(WS)	65	0.9077(59)	0.0462(3)	0.0462(3)	0.9308	0.0692
	Xiangxi(XX)	23	0.8696(20)	0.0435(1)	0.0807 (2)	0.8913	0.1087
	Zhaotong(ZT)	37	0.9730(36)	0.0207(1)	0.0000(0)	0.9865	0.0135
	Guizhouweining(GZWN)	25	0.9200(23)	0.0800(2)	0.0000(0)	0.9600	0.0400
	Yunling(YL)	50	1.0000(50)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Diqing(DQ)	48	1.0000(48)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Sanjiang(SJ)	39	1.0000(39)	0.0000(0)	0.0000(0)	1.0000	0.0000
	Dianzhong(DZ)	31	0.9355(29)	0.0323(1)	0.0323(1)	0.9516	0.0484
	Dengchuan(DC)	12	0.9167(11)	0.0833(1)	0.0000(0)	0.9583	0.0417
	Dehong(DH)	24	0.9583(23)	0.0417(1)	0.0000(0)	0.9792	0.0208
	Enshi(ES)	10	0.5000(5)	0.2000(2)	0.3000(3)	0.6000	0.4000
	Guangfeng(GF)	11	0.7273(8)	0.1818(2)	0.0909(1)	0.8182	0.1818
	Guanling(GL)	20	0.7000(14)	0.2500(5)	0.0500(1)	0.8250	0.1750
	Huangpi(HP)	18	0.6111(11)	0.3333(6)	0.0556(1)	0.7778	0.2222
	Ji'an(JA)	11	0.7273(8)	0.2727(3)	0.0000(0)	0.8636	0.1364
	Jiangcheng(JC)	41	0.9756(40)	0.0000(0)	0.0244(1)	0.9756	0.0244
	Jinghong(JH)	6	0.6667(4)	0.3333(2)	0.0000(0)	0.8333	0.1667
	Jinjiang(JJ)	13	0.4615(6)	0.3077(4)	0.2308(3)	0.6154	0.3846
	Leiqiong(LQ)	33	0.6667(22)	0.2424(8)	0.0909(3)	0.7879	0.2121
	Longlin(LL)	72	0.7631(53)	0.2083(15)	0.0556(4)	0.8403	0.1597
	Linnan(LN)	8	0.5000(4)	0.3750(3)	0.1250(1)	0.6875	0.3125
	Loudi(LD)	30	0.2667(8)	0.5333(16)	0.2000(6)	0.5333	0.4667
	Sinan(SN)	20	0.6000(12)	0.3500(7)	0.0500(1)	0.7750	0.2250
	Tiantai(TT)	30	0.4333(13)	0.5333(16)	0.0334(1)	0.7000	0.3000
	Weining(WN)	20	0.6000(12)	0.4000(8)	0.0000(0)	0.8000	0.2000
	Wenling(WL)	52	0.5000(26)	0.4038(21)	0.0962(5)	0.7019	0.2981
	Wuchuan(WC)	20	0.5500(11)	0.2500(5)	0.2000(4)	0.6750	0.3250
	Xiangnan(XN)	19	0.1580(3)	0.3158(6)	0.5263(10)	0.3158	0.6842
	Zhoushan(ZS)	37	0.2432(9)	0.4865(18)	0.2703(10)	0.4865	0.5135
Other regions	Burma	49	0.9388(46)	0.0612(3)	0.0000(0)	0.9694	0.0306
	Yakutian	5	0.8000(4)	0.0000(0)	0.2000(1)	0.8000	0.2000
Total		1702					

2.4. Introgression validation analysis

IQ-TREE v1.6.6 was used to construct phylogenetic tree for the introgressed regions of *NPBWR2* from banteng. A tree topology was constructed by the Neighbor-joining (NJ) method. Previous studies have demonstrated evidence of introgression from banteng into the genome of East Asian indicine (EAI) cattle. Based on this, we identified the introgression region by integrating PCR analysis of 34 breeds/populations with whole-genome resequencing of 29 breeds/populations, encompassing a total of 63 Chinese indigenous breeds.⁸ The tree was constructed using the merged sequences of introgressed segments of each Chinese indicine cattle according to the region (BTA13:53,650,000–53,840,000 bp) and homologous sequences of eight other bovine species. The phylogenetic tree was finalized and visualized using FigTree v1.4.3. Graphical elements, including branch colors, terminal shapes and text fonts, were optimized to produce a clear figure. The NJ phylogeny of EAI (brown samples) cattle supported the introgression of banteng in 60 EAI genomes.

2.5. Data processing and statistical analysis methods

The genotypic and allele frequencies were calculated directly from the observed genotypes in the analyzed breeds. Table 1 presents the specific allele and genotypic frequencies for the 63 Chinese indigenous breeds/populations and two populations from other regions.

3. Results and analysis

3.1. Phylogenomic validation of *NPBWR2* introgression from banteng

Our genomic analysis revealed introgression events in Chinese indicine cattle with 1,267 genes of banteng origin, including *NPBWR2* gene.⁸ To further confirm this introgressed region, a NJ phylogeny was constructed for this region from Chinese indicine cattle and other bovine species. We constructed a phylogenetic tree from a final set of 173 sequences representing eight bovine species.⁸ In the phylogenetic tree, the EAI haplotype clustered with banteng, indicating that this region was introgressed from banteng (Figure 1). This is consistent with the allele frequency data (Table 1), which shows the highest *NPBWR2* frequency in EAI cattle.

3.2. Detection of a missense mutation in the *NPBWR2* gene of Chinese cattle

NPBWR2 gene was searched for its introgressed region and a novel C-to-G mutation (NC_083880.1:c.14C>G) was identified within exon 5 of this gene from the BGVD. This mutation results in a threonine to serine (Thr5Ser) substitution (<http://animal.nwsuaf.edu.cn/code/index.php/BosVar>).¹³ The homozygous GG genotype also identified in 10 banteng individuals, indicating its banteng origin.

3.3. Genetic parameter analysis of NC_083880.1: c.14C>G of the *NPBWR2* gene

The distributions of the NC_083880.1: c.14C>G genotypes in Chinese indigenous cattle breeds/populations and two other populations are CC (n=1253), CG (n=324), and GG (n=125), with frequencies of 0.7362, 0.1904, and 0.0734 for each genotype, respectively (Figure 2). The proportions of the C and G allele are 0.8314 and 0.1686, respectively. The homozygous GG genotype in the Chinese indigenous cattle was found in ten individuals of banteng. Further confirming the origin of this mutation from banteng. Then we studied the geographical distribution of NC_083880.1: c.14C>G in 63 Chinese indigenous cattle breeds/populations, one population in Burma, and one banteng population, as shown in Figure 2. The results revealed that the frequency of allele G gradually increased from northern to southern China, whereas the frequency of allele C showed the opposite trend.

A total of 63 Chinese indigenous breeds/populations were used for genotyping *NPBWR2* mutation using PCR (34 breeds/populations) and whole genome resequencing (29 breeds/populations) methods. The allele frequency of G in the southern group of cattle was significantly greater than that in the

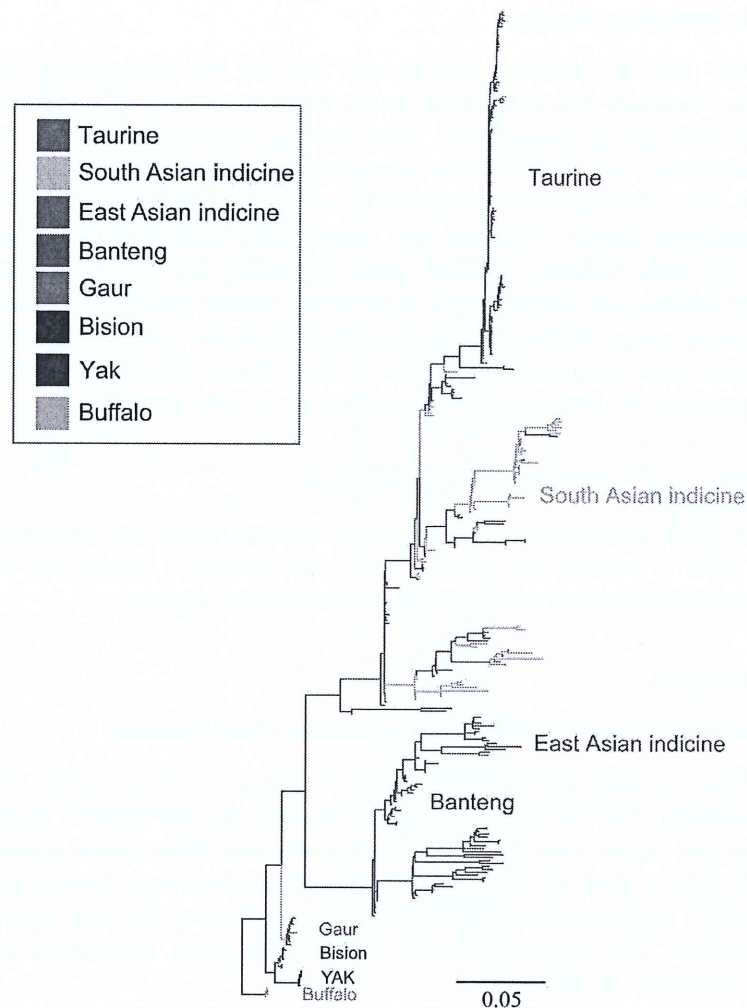


Figure 1. Phylogenetic tree constructed using the haplotype sequences from the BTA13:53,740,001–53,790,000 region, including *NPBWR2* gene, among representative bovine species. Haplotypes of East Asian indicine (EAI) cattle that are clustered with banteng (*Bos javanicus*) indicate banteng introgression into EAI cattle.

northern group. According to the geographical distribution, the frequency of allele G exceeded 0.50 in several southern Chinese cattle breeds including Xiangnan cattle (0.6842) and Zhongshan cattle (0.5135) (Table 1). The predominance of the allele G in these southern populations is consistent with adaptive introgression from banteng, facilitating the tropical or subtropical adaptation of indicine cattle.

The northern and the central regions unanimously presented CC genotype and did not harbor GG genotype. The presence of the GG genotype almost exclusively in the southern Chinese cattle. This pattern implies that the *NPBWR2* mutation was originated from banteng, possibly contributing to indicine cattle adaptation.

4. Discussion

Cattle are one of the world's most important domesticated species, primarily comprised of the two subspecies *B. taurus* and *B. indicus*. The two domesticated subspecies are believed to have diverged 200,000–300,000 years ago.⁷ The other bovine species including *B. gaurus*, *B. frontalis*, *B. grunniens*, and *B. javanicus* are distributed in and around China and are capable of hybridizing with the domestic cattle.^{12,18,21} *B. javanicus* (banteng) inhabit various regions of Southeastern Asia including parts of South China and crossbreed between banteng and Chinese indicine cattle has shaped the genetic material to the local cattle populations.¹²

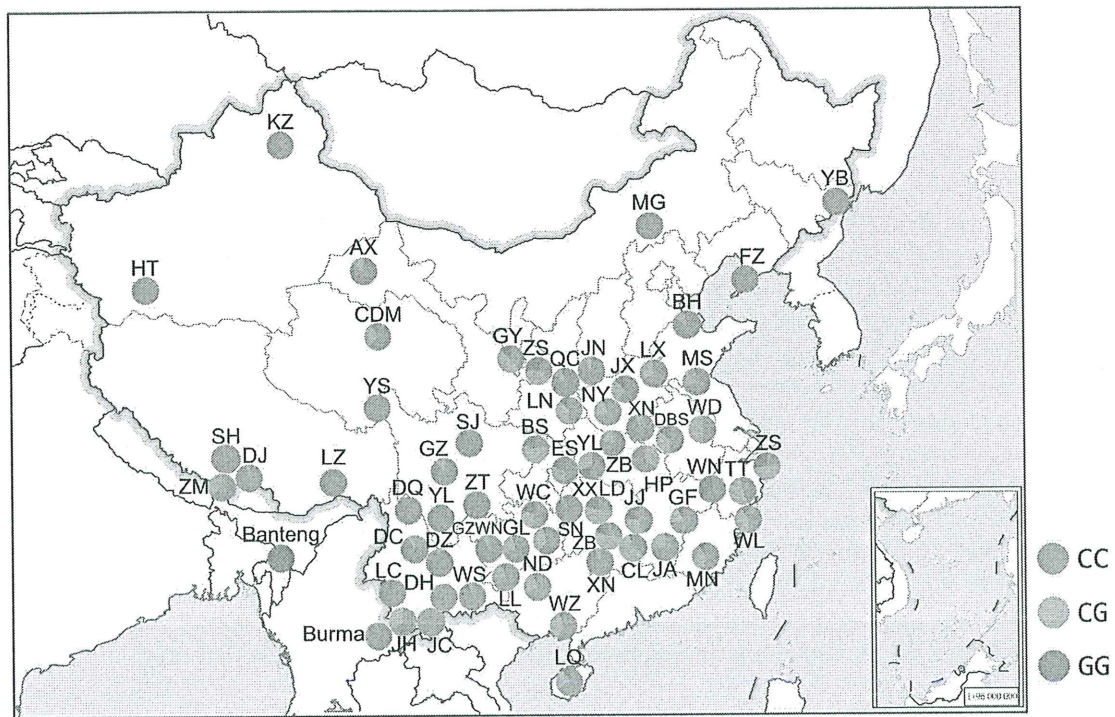


Figure 2. Geographic distribution of the missense mutation in the *NPBWR2* gene (NC_083880.1: c.14C>G).

Note:

Northern group:

CDM, Chaidamu; DJ, Dingjie; SH, Shigatse Humped; GZ, Ganzi; MG, Mongolian; HT, Hetian; YS, Yushu; ZM, Zhangmu; LZ, Linzhi; YB, Yanbian; KH, Kazakh; AX, Anxi; FZ, Fuzhou;

Central group:

ZS, Zaosheng; GY, Guyuan; JX, Jiaxian; BH, Bohai; ZB, Zaobei; JN, Jinnan; QC, Qinchuan; LX, Luxi; MS, Mengshan; NY, Nanyang; BS, Bashan;

Southern group:

YL, Yiling; LC, Lincang; DBS, Dabieshan; XN, Xianan; MN, Minnan; CL, Chaling; ND, Nandan; WN, Wannan;

WZ, Weizhou; WD, Wandong; WS, Wenshan; XX, Xiangxi; ZT, Zhaotong; GZWN, Guizhouweining; YL, Yunling; DQ, Diqing; SJ, Sanjiang; DZ, Dianzhong;

DC, Dengchuan; DH, Dehong; ES, Enshi; GF, Guangfeng; GL, Guanling; HP, Huangpi; JA, Jian; JC, Jiangcheng; JH, Jinghong; JJ, Jinjiang; LQ, Leiqiong; LL, Longlin; LN, Linnan; LD, Loudi; SN, Sinan; TT, Tiantai; WN, Weining; WL, Wenling; WC, Wuchuan; XN, Xiangnan; ZS, Zhoushan.

Neuropeptide B/W receptor-2 (*NPBWR2*) is a G protein-coupled receptor, which has been identified as an introgressed gene from Banteng in the Chinese indicine cattle.²² *NPBWR2* has been implicated in the regulation of feeding behavior, energy homeostasis, and neuroendocrine function and inflammatory pain modulation.^{23,24,25,26,27,28} In the current study, a missense mutation (NC_083880.1: c.14C>G) in Chinese indicine cattle has been identified by using BGVD. Here, we explored the allele frequency of this mutation in 1648 individuals belonging to 63 Chinese indigenous cattle breeds/populations using PCR amplification followed by Sanger sequencing and whole genome resequencing. The analysis revealed a strong geographical gradient, indicating that the G allele was most prevalent in the southern Chinese cattle populations. This pattern decreased toward the northern region where the ancestral C allele was predominant. The introgression of the *NPBWR2* gene from banteng into Chinese indicine cattle may have contributed to its enhanced adaptability to tropical or subtropical environments. Different studies have shown that neuropeptide W is involved in regulating food and energy metabolism,^{22,26} and appropriate *NPW* signaling is very important for the full expression of normal species-specific behavior under stress.^{23,29} Banteng habitats in the tropical rainforests and feeds on grass, bamboo, and leaves, making it highly adaptable to tropical or subtropical environments.⁸ These findings suggest that the *NPBWR2* variant exhibits regional specificity. Therefore, the introgression of *NPBWR2* may facilitate improved metabolic and feeding behavior in Chinese indicine cattle, which likely represents an introgression event followed by local adaptation from north to south China, ultimately promoting adaptation to the tropical and subtropical climates of Chinese indicine cattle.

Our previous research has found that a missense mutation (NC_083880.1: c.14C>G) replaces the original polar hydroxyl group (Thr) with a structurally similar polar hydroxyl group (Ser). As polar uncharged amino acids, serine and threonine are both canonical targets for phosphorylation. While the Thr-to-Ser substitution is considered conservative at the physicochemical level, threonine possesses an additional methyl group on its β -carbon. This structural distinction may introduce local steric constraints or alter side-chain orientation, potentially affecting docking geometry with kinase active sites.³¹ Notably, many kinases exhibit distinct substrate specificity between serine and threonine residues.³² Consequently, this missense mutation could result in one or more of the following functional consequences: 1) Altered post-phosphorylation conformational dynamics. Phosphorylation-induced conformational changes are a key regulatory mechanism for protein function. The Thr5Ser substitution may perturb the local structural equilibrium of the phosphorylated protein—either impairing its ability to revert to the native state or stabilizing non-canonical conformations—thereby modulating protein activity, interaction networks, and ultimately cellular output;³¹ 2) Modulated phosphorylation kinetics. Even if the mutated residue remains recognizable by cognate kinases, the substitution may fine-tune the catalytic efficiency of phosphorylation (either increased or decreased). Such kinetic changes could directly influence the amplitude and temporal dynamics of downstream signal transduction;³¹ 3) Rewired phosphorylation site specificity. The mutation may abrogate recognition by kinases that strictly require threonine at this position or, conversely, enable phosphorylation by serine-preferring kinases. Either scenario would reprogram the global phosphorylation landscape of the protein, with potential impacts on its regulation and functional partnerships.³³

5. Conclusion

In conclusion, our results revealed a clear geographical pattern in the distribution and frequency of the *NPBWR2* alleles in Chinese indicine cattle. The frequency of allele C gradually decreased from North China to the South China, while the allele G increased along the same gradient. The origin of the allele G shows strong evidence of introgression from banteng and the allelic pattern also reflects the introgression from banteng to Chinese indicine cattle population which rapidly promoted the adaptation to tropical and subtropical climates.³

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Author contribution

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Disclosure statement

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