The Journal of Agricultural Science

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Association analysis of *SSTR2* copy number variation with cattle stature and its expression analysis in Chinese beef cattle

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Animal Research Paper

Cite this article: Cheng J, Jiang R, Cao XK, Liu M, Huang YZ, Lan XY, Cao H, Lei CZ, Chen H (2019). Association analysis of *SSTR2* copy number variation with cattle stature and its expression analysis in Chinese beef cattle. *The Journal of Agricultural Science* **157**, 365–374. https://doi.org/10.1017/S0021859619000674

Received: 24 March 2019
Revised: 15 August 2019
Accepted: 9 September 2019
First published online: 26 September 2019

Key words:
Association analysis; cattle stature; copy number variations; mRNA expression; SSTR2

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Abstract

Copy number variations (CNVs), as an important source of genetic variation, can affect a wide range of phenotypes by diverse mechanisms. The somatostatin receptor 2 (*SSTR2*) gene plays important roles in cell proliferation and apoptosis. Recently, this gene was mapped to a CNV region, which encompasses quantitative trait loci of cattle economic traits including body weight, marbling score, etc. Therefore, *SSTR2* CNV may exhibit phenotypic effects on cattle growth traits. In the current study, distribution of *SSTR2* gene CNVs was investigated in six Chinese cattle breeds (XN, QC, NY, JA, LX and PN), and the results showed higher CNV polymorphisms in XN, QC and NY cattle. Next, association analysis between growth traits and *SSTR2* CNV was performed for XN, QC and NY cattle. In NY, individuals with fewer copies showed better performance than those with more copies. Further, the effects of *SSTR2* CNV on the *SSTR2* mRNA level were also investigated, but revealed no significant correlation in either muscle or adipose tissue of adult NY cattle. The results suggested the potential for use of *SSTR2* CNV as a marker for the molecular breeding of NY cattle.

Introduction

Copy number variation (CNV) is a variation in genomic sequence that ranges from 50 bp to 5 Mb. Compared with a reference sequence, CNV includes insertions, deletions and duplications (Mills *et al.*, 2011; MacDonald *et al.*, 2014). Numerous CNVs have been routinely identified using various genome analysis platforms, including single nucleotide polymorphism (SNP) genotyping platforms (Di Gerlando *et al.*, 2019), array comparative genomic hybridization (aCGH) (Zhang *et al.*, 2014) and next-generation sequencing (Xu *et al.*, 2017). These studies have been performed in humans (Altshuler *et al.*, 2010; Mills *et al.*, 2011), mice (Guryev *et al.*, 2008; Yalcin *et al.*, 2011), pigs (Wang *et al.*, 2013*a*, 2014), horses (Doan *et al.*, 2013; Kader *et al.*, 2016; Corbi-Botto *et al.*, 2019), cattle (Jiang *et al.*, 2013; Yang *et al.*, 2017*a*), goats (Fontanesi *et al.*, 2010; Liu *et al.*, 2018; Zhang *et al.*, 2019) and chickens (Wang *et al.*, 2010). Over the past decades, significant progress has been made in mapping SNPs and insertions/deletions (Indels), the lengths of which are much smaller than those of CNVs, but there is less comprehensive annotation of CNVs (Pang *et al.*, 2010). Although SNPs have a disadvantage in quantity, CNVs make up a higher proportion of genomes compared with SNP (Yang *et al.*, 2017*b*). Additionally, CNVs can have potential effects on phenotypic variation through various molecular mechanisms including gene interruption, gene fusion, gene dosage, position effects, unmasking of recessive alleles or functional polymorphisms, and transvection effects (Zhang *et al.*, 2009). Overall, the variations in copy number distributed in the genome also represent a major source of genetic and phenotypic variation among individuals (Sebat *et al.*, 2004; Beckmann *et al.*, 2007), and are associated with the occurrence of several diseases, especially some cancers (McCarroll and Altshuler, 2007).

Somatostatin receptor 2 (SSTR2), a seven-transmembrane-domain protein receptor, has two isoforms (SSTR2A and SSTR2B) which belong to the family of transmembrane G-protein coupled receptors (GPCRs) and play important roles in cell signal pathways by binding the somatostatin ligand. In detail, GPCRs include five members (SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5). In the 1990s, the five members were successfully cloned in humans (Yamada *et al.*, 1992*a*, *b*, 1993) and found to share DNA sequence coding for a transmembrane region (Heron *et al.*, 1993). Among these five somatostatin receptors, SSTR2 is mainly expressed in the cerebral cortex, the pituitary and adrenal glands in humans, and it was reported to exert anti-proliferative and pro-apoptotic effects by the negative regulation of the Wnt/β-catenin pathway (Buscail *et al.*, 1995; Chen *et al.*, 2009; Wang *et al.*, 2013*b*).

The bovine *SSTR2* gene is located at chr19: 58716920-58723781 (UMD_3.1.1), with an 1107 bp sequence that encodes a 368 amino acid protein. The *SSTR2* gene has been identified

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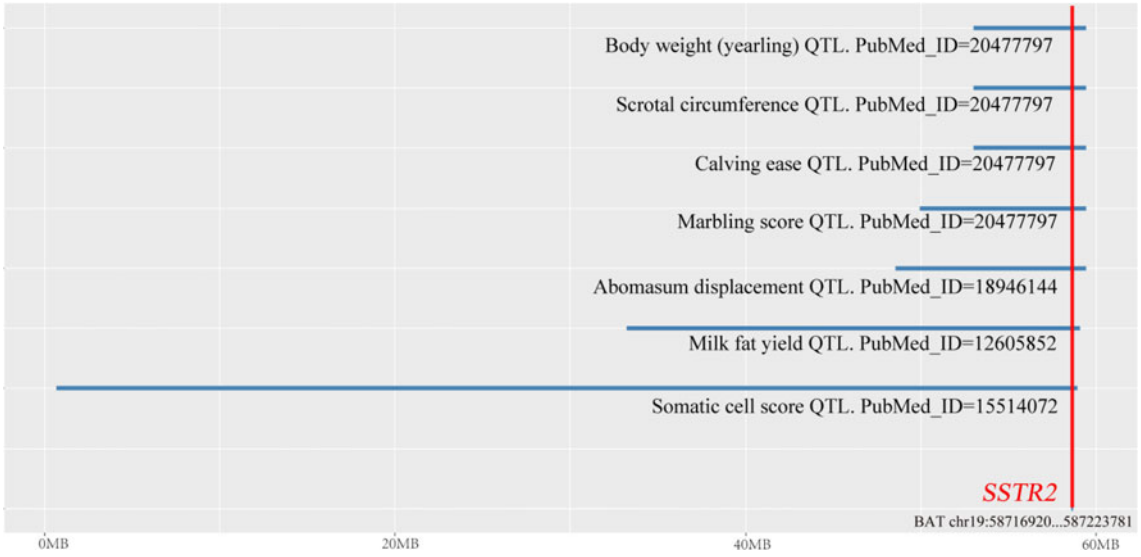

Fig. 1. Various QTLs associated with *SSTR2*. Colour online. Note: Using R software, data from Animal QTLdb and NCBI.

in important quantitative trait loci (QTLs) such as somatic cell score, milk fat yield, abomasum displacement, marbling score, calving ease, scrotal circumference and body weight (yearling) (Fig. 1) (Boichard *et al.*, 2003; Bennewitz *et al.*, 2004; Moemke *et al.*, 2008; McClure *et al.*, 2010). In a previous study, the *SSTR2* gene was mapped to a CNV region called CNVR317 in Chinese cattle by application of aCGH (Zhang *et al.*, 2014). The results suggested that *SSTR2* CNV is a phenotype-associated variation, but this has not been demonstrated conclusively. In the current study, quantitative polymerase chain reaction (qPCR) was used to detect the *SSTR2* CNV for six Chinese cattle breeds. Additionally, the significant effects of *SSTR2* CNV on the phenotype were identified in 431 individuals from three breeds.

Materials and methods

Study populations and trait records

The probes used in the previous aCGH experiment are shown in Fig. 2. In that study, eight individuals including three Qinchuan cattle, three Nanyang cattle and two Luxi cattle were selected to detect the CNV of *SSTR2* (Zhang *et al.*, 2014). In the current study, preliminary verification of CNVs was first performed on the representatives of six cattle breeds, and then the intergroup distributions of *SSTR2* CNVs were examined for six multi-variety panels. The selected cattle were Jian cattle (JA, $n = 30$), Qinchuan cattle (QC, $n = 30$), Nanyang cattle (NY, $n = 30$), Luxi cattle (LX, $n = 30$), Pinan cattle (PN, $n = 30$) and Xia'nan cattle (XN, $n = 30$), and were reared in Jiangxi, Shaanxi, Henan, Shandong, Henan and Henan provinces, respectively. Given the CNV polymorphisms of the six breeds, three populations, QC, NY and XN breeds, were scaled up for association analysis. The subject animals were weaned at 6 months old, fed *ad-libitum* on concentrated diet and maize–maize silage diet and given straw until about 2 years old. The animals used for association analysis were unrelated for at least the past three generations. Growth records of the XN, QC and NY animals were collected for association analysis (Gilbert *et al.*, 1993). In XN cattle ($n = 216$), the withers height, body weight, body oblique length, chest girth, hip width, paunch girth and cannon bone circumference were measured for cows and oxen (24 months old). In the QC breed ($n = 105$), withers height, body weight, body length, hip width, chest girth, chest width, chest depth, thurl width, hucklebone width and rump length were measured for adult cows at 2 and 3.5 years old. In NY cattle ($n = 110$), withers height, body weight, body oblique length, chest girth, hucklebone width and average daily gain for different growth periods (0, 6, 12, 18, 24 and 36 month(s) old) were determined for cows.

Genomic DNA and total RNA isolation

To perform expression profiling analysis of *SSTR2* gene, three adult NY cattle (24 months old) which exhibited no adverse health conditions were selected for tissue collection, including heart, liver, spleen, lung, kidney, skeletal muscle and adipose tissue. To make an association analysis between genotypes and expression, skeletal muscles and adipose tissue samples of adult NY cattle ($n = 23$) were collected for RNA and DNA isolation.

Genomic DNA from blood and tissue samples was isolated according to standard procedures (Sambrook *et al.*, 2001). The total RNA was extracted by Trizol reagent according to the manufacturer's instructions (TaKaRa, Japan). The RNA integrity was detected by agarose gel electrophoresis and RNA purity was determined by A260/A280. The synthesis of cDNA was performed using the PrimeScript RT reagent kit (TaKaRa, Japan). The diluted standard concentration of DNA and cDNA samples was 50 ng/μl and the samples were stored at −20 °C.

Determination of SSTR2 gene copy numbers

The copy number of *SSTR2* was detected by qPCR through comparison with the reference gene, ribonuclease P/MRP subunit p30 (*RPP30*) (Hindson *et al.*, 2011), widely recognized as a reference gene with two copies, and primers were designed using Primer 5 software (Table 1). The qPCR reactions were performed as described (Liu *et al.*, 2016). The standard curve method (using six serial dilution points) indicated similar amplification efficiencies of target and housekeeping genes. Finally, 431 animals including XN ($n = 216$), QC ($n = 105$) and NY ($n = 110$) cattle

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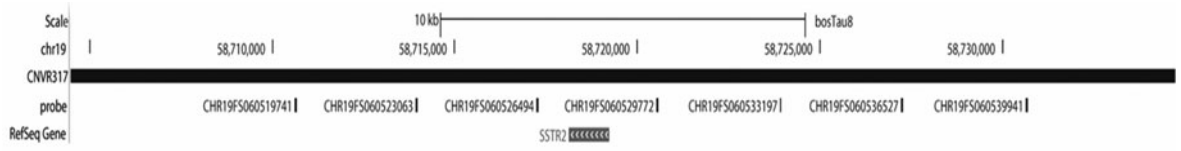

Fig. 2. Schematic diagram of the mapped aCGH probes for cattle *SSTR2* gene. Note: The *SSTR2* gene sequences were obtained from the cattle UCSC Genome (Bos Tau 4.0). CHR19FS060519741-Chr 19: 58710608–58710657; CHR19FS060523063-Chr 19: 58713930–58713980; CHR19FS060526494-Chr 19: 58717235–58717293; CHR19FS060529772-Chr 19: 58720513–58720562; CHR19FS060533197-Chr 19: 58723903–58723952; CHR19FS060536527-Chr 19: 58727233–58727283; CHR19FS060539941-Chr 19: 58730647–58730696.

Table 1. PCR primer sequences of the cattle *SSTR2* gene for qPCR in the current study

Gene	Primer number	Primers(5′–3′)	Fragments
SSTR2 – CNV	P1	F : CTCTTCGGTCTCAGTGGC	216
		R: CGGGATTTGTCCTGCTTA	
RPP30 – CNV	P2	F : TGCTTCCATTGTTTCCTGATGA	96
		R: TGGGACCAGGTTCCATGATC	
SSTR2 – mRNA	P3	F : TGCCAACCCTATCCTCTAT	121
		R: GTCCTGCTTACTGTCACTCC	
ACTB – mRNA	P4	F : GTCATCACCATCGGCAATGAG	84
		R: AATGCCGCAGGATTCCATG	
GAPDH – mRNA	P5	F : CGACTTCAACAGCGACACTCAC	119
		R: CCCTGTTGCTGTAGCCAAATTC	

were used for further analyses. The copy number was calculated according to $2^{-\Delta\Delta Ct}$, and data were rounded (Shi *et al.*, 2016).

The effects of SSTR2 copy number variations on gene expression

Expression profiling of *SSTR2* was analysed by qPCR in different tissues, including heart, liver, spleen, lung, kidney, skeletal muscle and adipose tissue. The actin beta (*ACTB*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) genes were selected as the reference genes (Olias *et al.*, 2014). The skeletal muscles and adipose tissue of the adult NY cattle ($n = 23$) were also subjected to analysis by qPCR, which was done the same way as used for the expression profiling. Primer information is listed in Table 1, and relative expression levels were calculated as $2^{-\Delta\Delta Ct}$.

Statistical analyses

A full statistical model was first used and then a reduced statistical model was used in the final analysis. The full statistical model contained fixed effects of copy number, age, sex, management group, birth season, farm and paternal effects. In the reduced statistical model, management group, birth season, farm and paternal effects were not used as factors, given their no significant effects on phenotypic variation. Thus, the reduced model was as follows:

$$Y_{ijkl} = u + A_i + S_j + \mathrm{CNV}_k + e_{ijkl}$$

where Y_{ijkl} represents the growth measurements, u is the overall mean of a given trait, A_i is the fixed effect due to i^{th} age, S_j is the fixed effect due to j^{th} sex, CNV_j is the fixed effect of k^{th} CNV type of *SSTR2*, and e_{ijkl} is the random residual error. The data for different species gave different parameters in the model (for XN, $A_i = 0$; for QC and NY, $S_j = 0$).

In the current study, CNVs were grouped into three classes: gain, copy number > 2; median, copy number = 2; loss, copy number < 2. These assessments allowed the classification of copy number measurements into discrete values of 'Gain', 'Loss' or 'Median', sometimes referred to as 'genotypes' of samples, as an extremely general form of CNV analysis (Xu *et al.*, 2013; Liu *et al.*, 2016; Yang *et al.*, 2017*b*). Raw copy-number measurements were classified into such general 'calls', which can lead to the loss of important information from the original data (McCarroll and Altshuler, 2007). Given the rarity of individuals with copy number ⩾6 compared to the individuals with lower copy number, copy numbers (0, 1, 2, 3, 4, 5 and ⩾6) were also fitted as genotype levels in the model for association analysis. The general linear model in SPSS (Inc., Chicago, IL, USA) was used for association analysis of *SSTR2* CNVs with growth traits. The proportion of phenotypic variation that was explained by CNV (R^2) was determined by partial correlation analysis using the reduced statistical model (Rauch *et al.*, 2010).

Results

Copy number variation polymorphisms of SSTR2 in six Chinese cattle breeds

In a previous study, the cattle *SSTR2* gene was mapped to CNVR317 using aCGH and the customized probes were finely dispersed in this region (Fig. 2). Signal alterations of five or more continuous probes were detected and defined the DNA segment as a CNV. Therefore, as shown in Fig. 3, the *SSTR2* CNV

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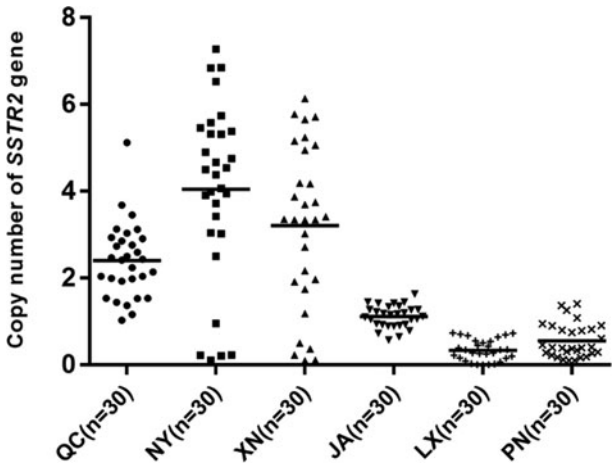

Fig. 3. Copy number distributions of the *SSTR2* in detecting panel calculated by $2^{-\Delta\Delta Ct}$. Note: QC ($n = 30$), Qinchuan cattle; NY ($n = 30$), Nanyang cattle; XN ($n = 30$), Xia'nan cattle; LX ($n = 30$), Luxi cattle; JA ($n = 30$), Jian cattle; PN ($n = 30$), Pinan cattle.

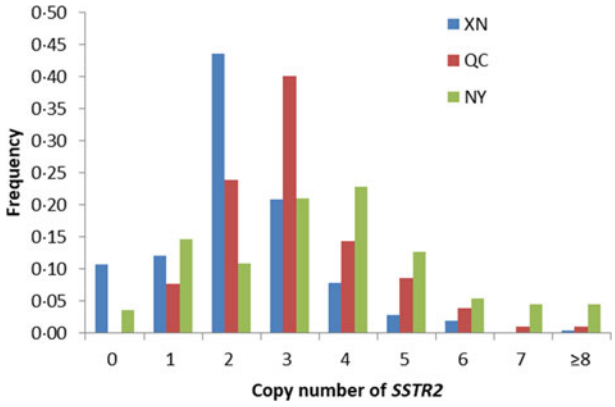

Fig. 4. Copy number frequencies of the *SSTR2* in large experimental groups calculated by $2^{-\Delta\Delta Ct}$. Colour online. Note: QC ($n = 106$), Qinchuan cattle; NY ($n = 111$), Nanyang cattle; XN ($n = 217$), Xia'nan cattle. Histograms show the frequency of individuals with different copy number. Copy numbers were rounded to the nearest integer.

polymorphisms were first validated by qPCR in 180 individuals from six Chinese cattle breeds (30 cattle per breed). In detail, XN, QC and NY cattle showed higher CNV polymorphisms in *SSTR2* loci than that of JA, LX and PN cattle. Based on this initial finding, the population sizes of QC, NY and XN were enlarged for further analysis. As illustrated in Fig. 4, *SSTR2* CNV polymorphisms exhibited a normal distribution. The highest frequency was observed for 2, 3 and 4 copies, respectively, in XN (94/216), QC (42/105) and NY (25/110) breeds, suggesting that the phenotypic effects of *SSTR2* CNVs may be highly variable in these three breeds.

Associations between SSTR2 copy number variations and growth traits in Chinese cattle

Three Chinese native cattle breeds, XN ($n = 216$), QC ($n = 105$) and NY cattle ($n = 110$), were used to analyse the association between *SSTR2* CNVs and growth traits. Table 2 shows an overview of the association of *SSTR2* CNVs with chest girth in NY cattle. In detail, the NY cattle with loss type of CNV had larger chest girths than those with the medium type ($P < 0.05$). From the data presented in Tables 3 and 4, no significant differences were detected in XN and QC cattle ($P > 0.05$). Next, the influence of different copy numbers on growth traits was analysed (Tables 5 and 6). The results presented in Table 5 were generally in agreement with the analysis of CNV types in NY cattle, in which copy numbers were significantly correlated with growth traits of chest girth ($P < 0.01$). Consistently, individuals with 0 copies had larger chest girths than those with more copies of the CNV. In XN cattle, the *SSTR2* copy numbers also had a significant effect on chest girth ($P < 0.01$), but the 4 copy was the advantageous variant type (Table 6). Above all, the data indicated that *SSTR2* CNV had effects on chest girth in NY and XN cattle. Additionally, as shown in Table 7, the *SSTR2* CNV had no effects on QC growth traits ($P > 0.05$). Notably, the CNV explained 6.4% variance of chest girth in the NY population.

Correlation analysis of SSTR2 copy number variation and mRNA expression level

The current study firstly investigated the correlation of mRNA level and *SSTR2* CNVs. First, expression profiling was performed for seven tissues, heart, liver, spleen, lung, kidney, skeletal muscle and adipose tissue samples from NY cattle. As shown in Fig. 5, the mRNA of *SSTR2* was widely expressed in adult cattle tissues. The highest abundance was observed in adipose tissue, suggesting that *SSTR2* has great effects on adipose tissue.

Because the quality of beef was an indicator in cattle breeding and the highest expression of *SSTR2* was seen in adipose tissue, muscle and adipose tissue were selected for sampling. The correlation of *SSTR2* CNVs with mRNA expression levels was analysed based on data from 23 adult NY cattle. It can be seen from the data presented in Table 8 that the copy numbers ranged from 1 to 3, with variation in mRNA expression both in muscle and adipose tissue, ranging from 0 to 6. However, no correlations were observed by analysis of these data ($P_{muscle} = 0.118$ and $P_{adipose} = 0.209$).

Discussion

With improvements in living standards, the demands for beef quantity and quality continue to grow. Marker-assisted selection (MAS) could compensate for traditional breeding methods to help meet the needs of consumers. For MAS, critical molecular markers need to be discovered and exhibit potent usage in genomics-assisted breeding programmes. Cao *et al.* (2018) reported that extracting causal genes underlying economic traits from QTL using the candidate gene method is a central strategy utilized in livestock breeding (Cao *et al.*, 2018). So far, common DNA sequence variations, such as SNP and Indels, have been widely used in genome-wide association studies. These approaches have allowed the identification of both critical and independent QTLs; these QTLs have enriched a larger variety of causal effects in the genome (Zheng *et al.*, 2017; Huang *et al.*, 2019).

In the current study, the *SSTR2* CNV was found to be located in important QTLs (Boichard *et al.*, 2003; Bennewitz *et al.*, 2004; Moemke *et al.*, 2008; McClure *et al.*, 2010), which implies that *SSTR2* CNV may be an important causal mutation for growth traits. In a previous CNV study by aCGH, cattle *SSTR2*, the full length of which is 6862 bp, was mapped to CNVR317 and located between probes CHR19FS060526494 and CHR19FS060529772. The probes in CNVR317 (chr19: 58598786-59376845, UMD

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Table 2. Association between *SSTR2* CNV types with cattle stature in NY cattle

	CNV types (LSM ± S.E.)			
Growth traits	Loss	Median	Gain	*P* value
Withers height	119.6 ± 0.76	121 ± 1.0	119.9 ± 0.53	0.497
Body weight	260 ± 3.6	256 ± 4.9	251 ± 2.5	0.125
Body oblique length	126.9 ± 0.84	127 ± 1.1	126.6 ± 0.59	0.817
Chest girth	157^{a} ± 1.1	153^{b} ± 1.5	155.1^{ab} ± 0.76	0.041
Hucklebone width	23.5 ± 0.27	23.5 ± 0.37	23.5 ± 0.19	0.999

Different letters in the same row mean significant difference (a, b: $P < 0.05$; A, B: $P < 0.01$). CNV, copy number variation; NY, Nanyang cattle; LSE, least square means; S.E., standard error.

Table 3. Association between *SSTR2* CNV types with cattle stature in XN cattle

	CNV types (LSM ± S.E.)			
Growth traits	Loss	Median	Gain	*P* value
Withers height	137.5 ± 0.65	136.9 ± 0.68	135.6 ± 0.71	0.087
Body weight	558 ± 7.9	562 ± 8.2	558 ± 8.5	0.851
Body oblique length	159 ± 1.0	160 ± 1.1	159 ± 1.1	0.501
Chest girth	199 ± 3.7	206 ± 3.8	210 ± 4.0	0.148
Hip width	140.7 ± 0.54	139.7 ± 0.56	139.2 ± 0.58	0.168
Paunch girth	217 ± 3.7	217 ± 2.1	214 ± 2.4	0.685
Cannon bone circumference	25 ± 1.9	24 ± 2.0	24 ± 2.1	0.940

CNV, copy number variation; XN, Xia'nan cattle; LSE, least square means; S.E., standard error.

Table 4. Association between *SSTR2* CNV types with cattle stature in QC cattle

	CNV types (LSM ± S.E.)			
Growth traits	Loss	Median	Gain	*P* value
Withers height	130 ± 2.0	129 ± 1.5	130.4 ± 0.99	0.671
Body weight	436 ± 21.9	431 ± 16.0	440 ± 10.7	0.797
Body length	139 ± 2.8	139 ± 2.0	140 ± 1.3	0.771
Hip width	127 ± 2.1	127 ± 1.5	128 ± 1.0	0.740
Chest girth	184 ± 3.6	182 ± 2.6	183 ± 1.7	0.854
Chest width	40 ± 1.6	40 ± 1.2	39.6 ± 0.78	0.977
Chest depth	65 ± 1.6	65 ± 1.2	64.8 ± 0.77	0.993
Thurl width	42 ± 1.3	44.1 ± 0.92	44.2 ± 0.61	0.253
Hucklebone width	24 ± 1.5	24 ± 1.1	23.5 ± 0.73	0.728
Rump length	45 ± 1.0	43.5 ± 0.76	44.1 ± 0.51	0.523

CNV, copy number variation; QC, Qinchuan cattle; LSE, least square means; S.E., standard error.

3.1.1) were finely dispersed, with a high density of about 30 probes per million bases. In the current study, specific primers were designed for *SSTR2* to validate the CNVs in six Chinese cattle breeds. The copy numbers in XN, QC and NY cattle were more dispersed than that in JA, LX and PN cattle. Accordingly, association analysis was conducted in XN, QC and NY cattle. Interestingly, individuals with low copy number showed better performance than the median and high copy number groups for chest girth in NY cattle. Although classifying copy number measurements as discrete values of 'Gain', 'Loss' or 'Median' in each sample is a standard practice in CNV analysis, this approach may lose some information that is present in the original measurements. Consequently, the copy numbers (0, 1, 2, 3, 4, 5 and ⩾6) were also fitted as fixed factors with seven levels in the

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Table 5. Association between *SSTR2* copy numbers with cattle stature in NY cattle

	Copy numbers (LSM ± S.E.)							
Growth traits	0	1	2	3	4	5	6	*P* value
Withers height	117 ± 1.5	120.4 ± 0.85	121 ± 1.0	121.0 ± 0.96	118.9 ± 0.77	121 ± 1.2	119 ± 1.3	0.118
Body weight	255 ± 7.4	262 ± 4.1	256 ± 4.9	261 ± 4.6	245 ± 3.7	252 ± 5.5	253 ± 6.5	0.057
Body oblique length	126^{ABbc} ± 1.6	127.3^{Aab} ± 0.91	127^{ABab} ± 1.1	130^{Aa} ± 1.0	123.9^{Bc} ± 0.82	127^{ABab} ± 1.2	128^{ABab} ± 1.4	0.001
Chest girth	160^{Aa} ± 2.2	157^{ABa} ± 1.2	153^{ABb} ± 1.5	158^{Aa} ± 1.4	153^{Bb} ± 1.1	158^{ABa} ± 1.7	154^{ABab} ± 1.9	0.007
Hucklebone width	24.7 ± 0.54	23.2 ± 0.30	23.6 ± 0.36	23.7 ± 0.34	23.4 ± 0.27	23.7 ± 0.41	23.5 ± 0.48	0.330

Different letters in the same row mean significantly difference (a, b: $P < 0.05$; A, B: $P < 0.01$). CNV, copy number variation; NY, Nanyang cattle; LSE, least square means; S.E., standard error.

Table 6. Association between *SSTR2* copy numbers with cattle stature in XN cattle

	Copy numbers (LSM ± S.E.)							
Growth traits	0	1	2	3	4	5	>= 6	*P* value
Withers height	137 ± 1.0	138 ± 1.0	136.99 ± 0.75	135.28 ± 0.888	136 ± 1.3	139 ± 2.1	135 ± 2.1	0.230
Body weight	546 ± 12.2	570 ± 12.2	568 ± 9.0	563 ± 10.6	551 ± 14.9	617 ± 25.2	545 ± 25.2	0.237
Body oblique length	159 ± 1.6	16 ± 1.6	160 ± 1.2	158 ± 1.4	160 ± 2.0	163 ± 3.4	156 ± 3.4	0.565
Chest girth	190^{Bc} ± 5.6	209^{Bb} ± 5.5	209^{Bb} ± 4.1	206^{Bb} ± 4.8	232^{Aa} ± 6.8	212^{ABabc} ± 11.4	208^{ABabc} ± 11.4	0.002
Hip width	140.5 ± 0.84	140.9 ± 0.84	140 ± 0.62	139 ± 0.73	141 ± 1.0	141 ± 1.7	140 ± 1.7	0.211
Paunch girth	205 ± 9.3	219 ± 4.0	217 ± 2.0	210 ± 3.0	221 ± 5.1	231 ± 8.3	207 ± 8.3	0.105
Cannon bone circumference	27 ± 3.0	23 ± 3.0	23 ± 2.2	23 ± 2.6	25 ± 3.7	24 ± 6.3	23 ± 6.3	0.973

Different letters in the same row mean significantly difference (a, b: $P < 0.05$; A, B: $P < 0.01$). CNV, copy number variation; XN, Xia'nan cattle; LSE, least square means; S.E., standard error.

Table 7. Association between *SSTR2* copy numbers with cattle stature in QC cattle

	Copy numbers (LSM ± SE)						
Growth traits	1	2	3	4	5	6	*P* value
Withers height	130 ± 2.0	129 ± 1.5	131 ± 1.1	131 ± 1.7	130 ± 2.1	125 ± 2.4	0.289
Body weight	435 ± 21.8	429 ± 16.0	447 ± 11.9	432 ± 18.1	437 ± 22.5	398 ± 26.6	0.540
Body length	138 ± 2.8	139 ± 2.0	140 ± 1.5	139 ± 2.3	140 ± 2.3	137 ± 3.4	0.921
Hip width	127 ± 2.1	127 ± 1.5	128 ± 1.1	128 ± 1.7	128 ± 2.1	123 ± 2.5	0.460
Chest girth	183 ± 3.5	182 ± 2.6	184 ± 1.9	182 ± 2.9	183 ± 3.6	176 ± 4.3	0.484
Chest width	40 ± 1.6	39 ± 1.2	39.9 ± 0.87	40 ± 1.3	39 ± 1.7	37 ± 1.9	0.698
Chest depth	64 ± 1.5	64 ± 1.1	65.5 ± 0.83	65 ± 1.3	62 ± 1.6	61 ± 1.9	0.094
Thurl width	42 ± 1.3	44.0 ± 0.93	44.2 ± 0.69	45 ± 1.0	44 ± 1.3	42 ± 1.5	0.467
Hucklebone width	24 ± 1.5	24 ± 1.1	23.8 ± 0.80	24 ± 1.2	21 ± 1.5	20 ± 1.8	0.184
Rump length	45 ± 1.0	43.5 ± 0.77	44.3 ± 0.57	43.8 ± 0.87	44 ± 1.1	43 ± 1.3	0.726

CNV, copy number variation; QC, Qinchuan cattle; LSE, least square means; SE, standard error.

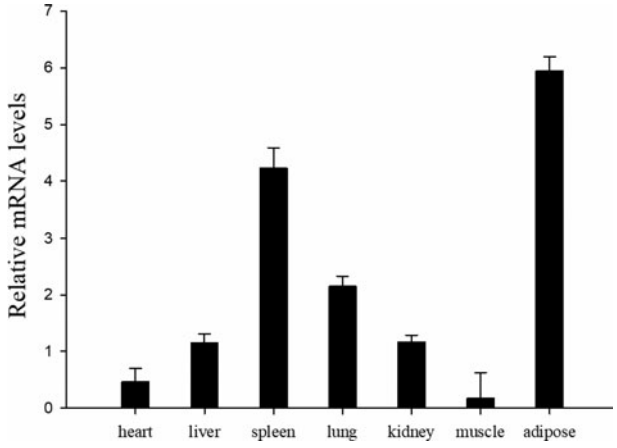

Fig. 5. Expression profiling of *SSTR2* in different tissues of adult NY cattle ($n = 3$). Note: Error bars represent the standard error (SE) ($n = 3$). The relative mRNA expression levels of *SSTR2* were normalized to *ACTB* and *GAPDH*.

model. Copy number with 0 levels had better chest girth than others in NY cattle ($P < 0.01$), which was consistent with the results above. *SSTR2* was reported in previous studies to have anti-proliferative effects on the Wnt/β-catenin pathway (Buscail *et al.*, 1995; Chen *et al.*, 2009; Wang *et al.*, 2013*b*), which may lead to better growth traits for individuals with low copy number. It can be seen from the current results that low copy number improved livestock growth traits, suggesting that the *SSTR2* CNV could be used as a molecular marker for NY cattle breeding. Next, CNV in the model (0, 1, 2, 3, 4, 5 and ⩾6) in XN cattle also exhibited a significant effect on chest girth. However, four copies were the advantageous type in XN. This is possibly because XN, a cultivated breed, has a crossbred genetic background, leading to the advantageous genotype of four copies. The results reveal that each breed has a specific genetic background which leads to various effects of different CNV polymorphisms. Overall, the

Table 8. Correlation analysis between the *SSTR2* CNVs and relative expression of *SSTR2* in adult muscle and adipose tissues in NY cattle ($n = 23$, F1–F23)

Individual	CNV	mRNA (muscle)	mRNA (adipose)
F1	1	1	0
F2	1	0	1
F3	2	1	3
F4	2	1	3
F5	2	0	3
F6	2	0	0
F7	1	0	1
F8	1	0	4
F9	2	1	0
F10	2	0	1
F11	1	0	1
F12	2	0	1
F13	2	1	2
F14	2	1	5
F15	1	0	1
F16	2	6	6
F17	3	3	2
F18	2	2	5
F19	2	0	1
F20	1	0	1
F21	3	2	3
F22	3	1	4
F23	2	1	4
P		0.118	0.209

CNV, copy number variation; NY, Nanyang cattle.

https://doi.org/10.1017/S0021859619000674 Published online by Cambridge University Press

CNV in *SSTR2* exerted a remarkable effect on chest girth, suggesting the quantity of meat can be improved.

Notably, the lower copy number had more positive effects on NY chest girth and intricate mechanisms may account for this unexpected result. Dosage effect is a key mechanism underlying the phenotypic effects of CNVs (Henrichsen *et al*., 2009; Karimi *et al*., 2018). To determine the potential mechanisms of dosage effect, expression analyses were performed and revealed that the mRNA of *SSTR2* was widely expressed in the different tissues, especially adipose. The quality of beef is an indicator of cattle breeding and the highest expression of *SSTR2* is detected in adipose tissue. Therefore, the correlation was analysed between *SSTR2* CNVs and its mRNA abundance in muscle and adipose tissue. Unfortunately, there were no significant correlations between *SSTR2* mRNA level and CNVs, suggesting that other complex interactions might contribute to the phenotypic effects of this CNV.

The variation of copy numbers may affect phenotypes through several different mechanisms. For example, (1) position effects of CNV: the variation of copy number can affect gene expression by influencing the relative position of a regulating factor and the gene. This effect can work even with a 1 Mb distance from the gene. (2) Fusion effects of CNV: the CNV may lead to the generation of fusion genes, which may have a new function. (3) Copy number variation in an encoding region will change the protein structure domain, thus affecting the structure and function of the protein (Hollox and Hoh, 2014). (4) A variation of copy numbers can also lead to the deletion of dominant alleles, which have inhibitory effects on recessive alleles, exposing a latent gene and resulting in the mutant phenotype (Beckmann *et al*., 2007).

Currently, there is keen interest in the identification of genetic loci that lead to livestock trait variations. Often, candidate gene methods are based on rudimentary knowledge about gene function or some presumed effects of candidate causal variants, which do not provide comprehensive mechanistic understanding (Karim *et al*., 2011). However, in recent decades, advances in integrative omics technologies such as genomics, transcriptomics, proteomics and metabolomics have begun to make accurate animal breeding possible at an extraordinarily detailed molecular level (Ritchie *et al*., 2015; Karczewski and Snyder, 2018). The current results are preliminary and further investigations should provide a mechanistic understanding of the genetic causality of *SSTR2* CNV.

Conclusion

This is the first analysis of the distribution of *SSTR2* CNV in six Chinese native cattle breeds. The association analysis of *SSTR2* CNV and phenotypic traits indicated that the *SSTR2* CNV can be used as a molecular marker for NY cattle breeding programmes.

Financial support. The current work was supported by the National Natural Science Foundation of China (no. 31772574), the Program of National Beef Cattle and Yak Industrial Technology System (CARS-37), Science and Technology Plan Project of Yangling Demonstration Area in 2018.

Conflict of interest. None.

Ethical standards. The current experiment was approved by the Northwest A&F University Ethics Committee.

References

Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Bonnen PE, de Bakker PIW, Deloukas P, Gabriel SB, Gwilliam R, Hunt S, Inouye M, Jia X, Palotie A, Parkin M, Whittaker P, Chang K, Hawes A, Lewis LR, Ren Y, Wheeler D, Muzny DM, Barnes C, Darvishi K, Hurles M, Korn JM, Kristiansson K, Lee C, McCarroll SA, Nemesh J, Keinan A, Montgomery SB, Pollack S, Price AL, Soranzo N, Gonzaga-Jauregui C, Anttila V, Brodeur W, Daly MJ, Leslie S, McVean G, Moutsianas L, Nguyen H, Zhang Q, Ghori MJR, McGinnis R, McLaren W, Takeuchi F, Grossman SR, Shlyakhter I, Hostetter EB, Sabeti PC, Adebamowo CA, Foster MW, Gordon DR, Licinio J, Manca MC, Marshall PA, Matsuda I, Ngare D, Wang VO, Reddy D, Rotimi CN, Royal CD, Sharp RR, Zeng C, Brooks LD, McEwen JE and Int HapMap C (2010) Integrating common and rare genetic variation in diverse human populations. *Nature* **467**, 52–58.

Beckmann JS, Estivill X and Antonarakis SE (2007) Copy number variants and genetic traits: closer to the resolution of phenotypic to genotypic variability. *Nature Reviews Genetics* **8**, 639–646.

Bennewitz J, Reinsch N, Guiard V, Fritz S, Thomsen H, Looft C, Kuhn C, Schwerin M, Weimann C, Erhardt G, Reinhardt F, Reents R, Boichard D and Kalm E (2004) Multiple quantitative trait loci mapping with cofactors and application of alternative variants of the false discovery rate in an enlarged granddaughter design. *Genetics* **168**, 1019–1027.

Boichard D, Grohs C, Bourgeois F, Cerqueira F, Faugeras R, Neau A, Rupp R, Amigues Y, Boscher MY and Leveziel H (2003) Detection of genes influencing economic traits in three French dairy cattle breeds. *Genetics Selection Evolution* **35**, 77–101.

Buscail L, Esteve JP, Saint-Laurent N, Bertrand V, Reisine T, O'Carroll AM, Bell GI, Schally AV, Vaysse N and Susini C (1995) Inhibition of cell proliferation by the somatostatin analogue RC-160 is mediated by somatostatin receptor subtypes SSTR2 and SSTR5 through different mechanisms. *Proceedings of the National Academy of Sciences USA* **92**, 1580–1584.

Cao XK, Huang YZ, Ma YL, Cheng J, Qu ZX, Ma Y, Bai YY, Tian F, Lin FP and Ma YL (2018) Integrating CNVs into meta-QTL identified GBP4 as positional candidate for adult cattle stature. *Functional & Integrative Genomics* **18**, 559–567.

Chen JS, Liang QM, Li HS, Yang J, Wang S and Long JW (2009) Octreotide inhibits growth of colonic cancer SW480 cells by modulating the Wnt/β-catenin pathway. *Pharmazie: An International Journal of Pharmaceutical Sciences* **64**, 126–131.

Corbi-Botto CM, Morales-Durand H, Zappa ME, Sadaba SA, Peral-Garcia P, Giovambattista G and Diaz S (2019) Genomic structural diversity in Criollo Argentino horses: analysis of copy number variations. *Gene* **695**, 26–31.

Di Gerlando R, Sardina MT, Tolone M, Sutera AM, Mastrangelo S and Portolano B (2019) Genome-wide detection of copy-number variations in local cattle breeds. *Animal Production Science* **59**, 815–822.

Doan R, Cohen N, Harrington J, Veazey K, Juras R, Cothran G, McCue ME, Skow L and Dindot SV (2013) Identification of copy number variants in horses. *Genome Research* **22**, 899–907.

Fontanesi L, Martelli PL, Beretti F, Riggio V, Dall'Olio S, Colombo M, Casadio R, Russo V and Portolano B (2010) An initial comparative map of copy number variations in the goat (*Capra hircus*) genome. *Bmc Genomics* **11**, 639.

Gilbert R, Bailey D and Shannon N (1993) Linear body measurements of cattle before and after 20 years of selection for postweaning gain when fed two different diets. *Journal of Animal Science* **71**, 1712–1720.

Guryev V, Saar K, Adamovic T, Verheul M, Van Heesch SAAC, Cook S, Pravenec M, Aitman T, Jacob H, Shull JD, Hubner N and Cuppen E (2008) Distribution and functional impact of DNA copy number variation in the rat. *Nature Genetics* **40**, 538–545.

Henrichsen CN, Chaignat E and Reymond A (2009) Copy number variants, diseases and gene expression. *Human Molecular Genetics* **18**, R1–R8.

Heron I, Thomas F, Dero M, Gancel A, Ruiz J, Schatz B and Kuhn J (1993) Pharmacokinetics and efficacy of a long-acting formulation of the new somatostatin analog BIM 23014 in patients with acromegaly. *The Journal of Clinical Endocrinology & Metabolism* **76**, 721–727.

Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, Kitano TK, Hodel MR, Petersen JF, Wyatt PW, Steenblock ER, Shah PH, Bousse LJ, Troup CB, Mellen JC, Wittmann DK, Erndt NG,

https://doi.org/10.1017/S0021859619000674 Published online by Cambridge University Press

Cauley TH, Koehler RT, So AP, Dube S, Rose KA, Montesclaros L, Wang S, Stumbo DP, Hodges SP, Romine S, Milanovich FP, White HE, Regan JF, Karlin-Neumann GA, Hindson CM, Saxonov S and Colston BW (2011) High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry* **83**, 8604–8610.

Hollox EJ and Hoh BP (2014) Human gene copy number variation and infectious disease. *Human Genetics* **133**, 1217–1233.

Huang H, Cao J, Guo G, Li X, Wang Y, Yu Y, Zhang S, Zhang Q and Zhang Y (2019) Genome-wide association study identifies QTLs for displacement of abomasum in Chinese Holstein cattle. *Journal of Animal Science* **97**, 1133–1142.

Jiang L, Jiang J, Yang J, Liu X, Wang J, Wang H, Ding X, Liu J and Zhang Q (2013) Genome-wide detection of copy number variations using high-density SNP genotyping platforms in Holsteins. *BMC Genomics* **14**, article no. 131. doi: https://doi.org/10.1186/1471-2164-14-131

Kader A, Liu X, Dong K, Song S, Pan J, Yang M, Chen X, He X, Jiang L and Ma Y (2016) Identification of copy number variations in three Chinese horse breeds using 70K single nucleotide polymorphism BeadChip array. *Animal Genetics* **47**, 560–569.

Karczewski KJ and Snyder MP (2018) Integrative omics for health and disease. *Nature Reviews Genetics* **19**, 299–310.

Karim L, Takeda H, Lin L, Druet T, Arias JA, Baurain D, Cambisano N, Davis SR, Farnir F, Grisart B, Harris BL, Keehan MD, Littlejohn MD, Spelman RJ, Georges M and Coppieters W (2011) Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature. *Nature Genetics* **43**, 405–413.

Karimi K, Esmailizadeh A, Wu DD and Gondro C (2018) Mapping of genome-wide copy number variations in the Iranian indigenous cattle using a dense SNP data set. *Animal Production Science* **58**, 1192–1200.

Liu M, Li B, Huang Y, Yang M, Lan X, Lei C, Qu W, Bai Y and Chen H (2016) Copy number variation of bovine MAPK10 modulates the transcriptional activity and affects growth traits. *Livestock Science* **194**, 44–50.

Liu M, Zhou Y, Rosen BD, Van Tassell CP, Stella A, Tosser-Klopp G, Rupp R, Palhière I, Colli L, Sayre B, Crepaldi P, Fang L, Mészáros G, Chen H and Liu GE and the ADAPTmap Consortium (2018) Diversity of copy number variation in the worldwide goat population. *Heredity* **122**, 636–646.

MacDonald JR, Ziman R, Yuen RKC, Feuk L and Scherer SW (2014) The database of genomic variants: a curated collection of structural variation in the human genome. *Nucleic Acids Research* **42**, D986–D992.

McCarroll SA and Altshuler DM (2007) Copy-number variation and association studies of human disease. *Nature Genetics* **39**(suppl), S37-S42.

McClure MC, Morsci NS, Schnabel RD, Kim JW, Yao P, Rolf MM, McKay SD, Gregg SJ, Chapple RH, Northcutt SL and Taylor JF (2010) A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle. *Animal Genetics* **41**, 597–607.

Mills RE, Walter K, Stewart C, Handsaker RE, Chen K, Alkan C, Abyzov A, Yoon SC, Ye K, Cheetham RK, Chinwalla A, Conrad DF, Fu Y, Grubert F, Hajirasouliha I, Hormozdiari F, Iakoucheva LM, Iqbal Z, Kang S, Kidd JM, Konkel MK, Korn J, Khurana E, Kural D, Lam HYK, Leng J, Li R, Li Y, Lin CY, Luo R, Mu XJ, Nemesh J, Peckham HE, Rausch T, Scally A, Shi X, Stromberg MP, Stuetz AM, Urban AE, Walker JA, Wu J, Zhang Y, Zhang ZD, Batzer MA, Ding L, Marth GT, McVean G, Sebat J, Snyder M, Wang J, Ye K, Eichler EE, Gerstein MB, Hurles ME, Lee C, McCarroll SA and Korbel JO and 1000 Genomes Project (2011) Mapping copy number variation by population-scale genome sequencing. *Nature* **470**, 59–65.

Moemke S, Scholz H, Doll K, Rehage J and Distl O (2008) Mapping quantitative trait loci for left-sided displacement of the abomasum in German Holstein dairy cows. *Journal of Dairy Science* **91**, 4383–4392.

Olias P, Adam I, Meyer A, Scharff C and Gruber AD (2014) Reference genes for quantitative gene expression studies in multiple avian species. *PLoS ONE* **9**, article no. e99678. doi: 10.1371/journal.pone.0099678

Pang AW, MacDonald JR, Pinto D, Wei J, Rafiq MA, Conrad DF, Park H, Hurles ME, Lee C, Venter JC, Kirkness EF, Levy S, Feuk L and Scherer SW (2010) Towards a comprehensive structural variation map of an individual human genome. *Genome Biology* **11**, article no. R52. doi: https://doi.org/10.1186/gb-2010-11-5-r52

Rauch F, Lalic L, Roughley P and Glorieux FH (2010) Relationship between genotype and skeletal phenotype in children and adolescents with osteogenesis imperfecta. *Journal of Bone and Mineral Research* **25**, 1367–1374.

Ritchie MD, Holzinger ER, Li R, Pendergrass SA and Kim D (2015) Methods of integrating data to uncover genotype–phenotype interactions. *Nature Reviews Genetics* **16**, 85–97.

Sambrook J, Russell D and Russell D (2001) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi MY, Navin N, Lucito R, Healy J, Hicks J, Ye K, Reiner A, Gilliam TC, Trask B, Patterson N, Zetterberg A and Wigler M (2004) Large-scale copy number polymorphism in the human genome. *Science* **305**, 525–528.

Shi T, Xu Y, Yang M, Huang Y, Lan X, Lei C, Qi X, Yang X and Chen H (2016) Copy number variations at LEPR gene locus associated with gene expression and phenotypic traits in Chinese cattle. *Animal Science Journal* **87**, 336–343.

Wang X, Nahashon S, Feaster TK, Bohannon-Stewart A and Adefope N (2010) An initial map of chromosomal segmental copy number variations in the chicken. *BMC Genomics* **11**, article no. 351. doi: https://doi.org/10.1186/1471-2164-11-351

Wang J, Wang H, Jiang J, Kang H, Feng X, Zhang Q and Liu JF (2013*a*) Identification of genome-wide copy number variations among diverse pig breeds using SNP genotyping arrays. *PLoS ONE* **8**, article no. e68683. doi: 10.1371/journal.pone.0068683

Wang S, Bao Z, Liang QM, Long JW, Xiao ZS, Jiang ZJ, Liu B, Yang J and Long ZX (2013*b*) Octreotide stimulates somatostatin receptor-induced apoptosis of SW480 colon cancer cells by activation of glycogen synthase kinase-3*β*, A Wnt/*β*-catenin pathway modulator. *Hepato-gastroenterology* **60**, 1639–1646.

Wang J, Jiang J, Wang H, Kang H, Zhang Q and Liu JF (2014) Enhancing genome-wide copy number variation identification by high density array CGH using diverse resources of pig breeds. *PLoS ONE* **9**, article no. e87571. doi: 10.1371/journal.pone.0087571

Xu Y, Zhang L, Shi T, Zhou Y, Cai H, Lan X, Zhang C, Lei C and Chen H (2013) Copy number variations of MICAL-L2 shaping gene expression contribute to different phenotypes of cattle. *Mammalian Genome* **24**, 508–516.

Xu Y, Jiang Y, Shi T, Cai H, Lan X, Zhao X, Plath M and Chen H (2017) Whole-genome sequencing reveals mutational landscape underlying phenotypic differences between two widespread Chinese cattle breeds. *PLoS ONE* **12**, article no. e0183921. doi: 10.1371/journal.pone.0183921

Yalcin B, Wong K, Agam A, Goodson M, Keane TM, Gan X, Nellaker C, Goodstadt L, Nicod J, Bhomra A, Hernandez-Pliego P, Whitley H, Cleak J, Dutton R, Janowitz D, Mott R, Adams DJ and Flint J (2011) Sequence-based characterization of structural variation in the mouse genome. *Nature* **477**, 326–329.

Yamada Y, Post SR, Wang K, Tager HS, Bell GI and Seino S (1992*a*) Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. *Proceedings of the National Academy of Sciences USA* **89**, 251–255.

Yamada Y, Reisine T, Law SF, Ihara Y, Kubota A, Kagimoto S, Seino M, Seino Y, Bell GI and Seino S (1992*b*) Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase. *Molecular Endocrinology* **6**, 2136–2142.

Yamada Y, Kagimoto S, Kubota A, Yasuda K, Masuda K, Someya Y, Ihara Y, Li Q, Imura H, Seino S and Y S (1993) Cloning, functional expression and pharmacological characterization of a fourth (hSSTR4) and a fifth (hSSTR5) human somatostatin receptor subtype. *Biochemical and Biophysical Research Communications* **195**, 844–852.

Yang L, Xu L, Zhu B, Niu H, Zhang W, Miao J, Shi X, Zhang M, Chen Y, Zhang L, Gao X, Gao H, Li L, Liu GE and Li J (2017*a*) Genome-wide analysis reveals differential selection involved with copy number variation in diverse Chinese cattle. *Scientific Reports* **7**, article no. 14299. doi: https://doi.org/10.1038/s41598-017-14768-0

Yang M, Lv J, Zhang L, Li M, Zhou Y, Lan X, Lei C and Chen H (2017*b*) Association study and expression analysis of CYP4A11 gene copy number variation in Chinese cattle. *Scientific Reports* **7**, article no. 46599. doi: 10.1038/srep46599

Zhang F, Gu W, Hurles ME and Lupski JR (2009) Copy number variation in human health, disease, and evolution. *Annual Review of Genomics and Human Genetics* **10**, 451–481.

Zhang L, Jia S, Yang M, Xu Y, Li C, Sun J, Huang Y, Lan X, Lei C, Zhou Y, Zhang C, Zhao X and Chen H (2014) Detection of copy number variations and their effects in Chinese bulls. *BMC Genomics* **15**, article no. 480. doi: https://doi.org/10.1186/1471-2164-15-480

Zhang RQ, Wang JJ, Zhang T, Zhai HL and Shen W (2019) Copy-number variation in goat genome sequence: a comparative analysis of the different litter size trait groups. *Gene* **696**, 40–46.

Zheng X, Kuang Y, Lv W, Cao D, Sun Z, Jin W and Sun X (2017) Quantitative trait loci for morphometric traits in multiple families of common carp (*Cyprinus carpio*). *Science China Life Sciences* **60**, 287–297.