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Full Length Research Paper

A novel polymorphism of resistin gene and its association with meat quality traits in Chinese *Bos taurus*

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Searching for candidate gene polymorphisms and their relationship with meat quality traits is an important issue for *Bos taurus* industry. In this study, we evaluated polymorphism of resistin (RETN) gene involved in energy metabolism. Using the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) technology, a novel missense substitution single-nucleotide polymorphism (SNP) g.2528T>C was identified in the exon 2 region of the RETN gene. Allele frequencies, gene heterozygosity, effective allele number and polymorphism information content of the bovine RETN SNP in six populations were investigated and calculated by the χ^2 test. The distribution of the polymorphism from the studied six *B. taurus* breeds was not uniform. Our results suggested that Qinchuan, Luxi and Luxi × Simmental were not in Hardy-Weinberg equilibrium. Moreover, the polymorphism and its association with meat quality traits were analyzed in 369 Qinchuan and 73 Nanyang individuals. The analyzed SNP in the RETN revealed a significant association with marbling and intramuscular fat ($p < 0.05$). We observed that TT genotype was associated with a heightened marbling score when compared with the TC and CC in Qinchuan and Nanyang breeds. In Qinchuan breed, we also found that TT genotype was associated with an increased intramuscular fat content when compared with the TC. Such associations were not observed on backfat thickness, loin-muscle area and loin-muscle depth. This study showed that polymorphism of the RETN gene is potentially associated with *B. taurus* meat quality traits.

Key words: *Bos taurus*, meat quality traits, polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), resistin gene, single nucleotide polymorphism (SNP).

INTRODUCTION

An integrated map of the cattle genome, which has been constructed since 1983 by Beckmann and Soller, supplies information about numerous chromosome regions (quantitative trait loci, QTL), presumably containing putative causative polymorphisms affecting variability of important productive traits. A majority of the QTLs deposited in the *Bos taurus* QTL database is associated with meat quality traits (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index). Studies on the genetic background of meat quality traits, like marbling score, intramuscular fat content and meat tenderness variability in *B. taurus* are also interesting from the point of view of breed improvement and conservation, since the molecular technology such as the detection of QTLs and single nucleotide polymorphisms (SNPs) are considered as useful methods in the selection and production of excellent breeds of animals.

The number of articles reporting an association between gene polymorphism and meat quality traits has

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recently increased, also in case of studies on *B. taurus*. It was shown that a well known functional polymorphism of the insulin growth factor 2 (IGF2) gene, influences meatiness, backfat thickness as well porcine (Van den et al., 2008) in cattle in many reports. In order to solve these problems, a number of techniques have been used, but the most conventional one is polymerase chain reaction - single strand conformation polymorphism (PCR-SSCP), which was found to be most convenient, accurate and more sophisticated. Similarly, hundreds of genes were selected for the research. Among candidate genes for meat quality traits, an important group consists of genes encoding proteins involved in energy metabolism, e.g., adipokines, cellular respiratory system elements, proteins regulating the process of food intake or factors controlling adipocyte proliferation and differentiation. There are genes whose polymorphism in relation to cattle meat quality traits has not been studied extensively, for example resistin (RETN). The gene coding for RETN is a 12.5 kDa protein, located at chromosome location 19p13.3 (Steppan et al., 2001a). RETN, an adipokine also known as a cysteine-rich adipose tissue-specific secretory factor (ADSF) or a cysteine-rich secreted protein found in inflammatory zone3 (FIZZ3) (Holcomb et al., 2000), is a member of resistin-like molecules (RELMs) (Steppan et al., 2001b). In original studies, RETN has been reported to be a tissue specific secretory factor in rodents (Steppan et al., 2001a; Kim et al., 2001), highly expressed in adipose tissue and participates in many metabolic pathways and inflammatory responses. RETN is reported to be associated with insulin resistance *in vivo* and *in vitro*. It has also been shown to influence insulin sensitivity in various tissues (Moon et al., 2003; Satoh et al., 2004). The mechanism is that it serves as a signaling molecule between the energy storage organ, adipose tissue and the principal insulin-responsive organs, liver, muscle and fat (Boden, 1997) and hence considered to be a major contributor to insulin resistance in obesity (Steppan et al., 2001a). In addition to altering insulin sensitivity, RETN has been shown to cause disturbances in glucose metabolism (Michael et al., 2003). Animal experiments have shown that RETN causes disorders in glucose metabolism and peripheral insulin resistance (Keller, 2006; Wolf, 2004). Several SNPs have been found in the RETN promoter, intron and 3'UTR (un-translated region) regions. RETN polymorphisms have also been found to be associated with other obesity-related phenotypes. An association has been found with blood pressure (Osawa et al., 2007), insulin resistance (Bouchard et al., 2004), obesity (Pistilli et al., 2007) and type 2 diabetes (Osawa et al., 2004).

Taking into consideration the chromosome localization of RETN gene within QTL regions for meat quality traits, as well as the function of the encoded proteins in energy metabolism, this study aimed to identify polymorphism in RETN and to evaluate the association between them and meat quality traits in selected Chinese *B. taurus* breeds.

MATERIALS AND METHODS

DNA samples and data collection

Preliminary screening for polymorphisms of the studied gene, RETN, was performed in a multibreed panel. A total of 694 adult animals were randomly selected from breeding populations and used to analyze the RETN allelic frequencies, including Qinchuan, QC (n = 369), Luxi, LX (n = 64), Nanyang, NY (n = 73), Jiaxian, JX (n = 68), Xia'nan, XN (n = 67), Luxi and Simmental cross breeding, LS (n = 53). Based on high polymorphism content (PIC), only 515 animals were selected for the association analysis with meat quality traits, including 442 animals of QC breed and 73 animals of NY breed. Moreover, QC cattle used for association study, represented two different age stages (18 and 24 months old). The following meat quality traits were measured by ultrasound: BF - backfat thickness, LMA - loin-muscle area, LMD - loin-muscle depth, MAR - marbling score and IMF - intramuscular fat content.

Genomic DNA samples were obtained from the 694 animals aforementioned. DNA samples were extracted from leukocytes and tissue samples using a standard phenol-chloroform protocol (Mullenbach et al., 1989), which was used for PCR-SSCP analysis.

PCR -SSCP and DNA sequencing

According to the sequence of the Bovine RETN gene (Gene Bank accession no. AY_618903) PCR primers (F:5' – CCAACCCA ACGCCAATCT - 3' and R: 5' – AACGGAGTTCTGTACCTACC - 3') for the RETN gene was designed using the Primer 5.0 tool and synthesized by Sirui-Jin Company in Shanghai, in order to amplify a 338 bp fragment of exon 2 of RETN gene spanning the g.2528T>C substitution. The PCR mix consist of 50 ng DNA template, 0.3 mM of each primer, 7.5 uL 2 x *Taq* polymerase and 5.9 µL ddH_2O. The total volume of each sample was 15 µL. The amplification was performed in a T gradient thermocycler. The PCR conditions were: 95°C/5 min (preliminary denaturation), 34 cycles of 94°C/30 s (denaturation), 57°C/35 s of primer annealing (temperature specific for single analyzed fragment), 72°C/35 s (extension) and 72°C/10 min of final extension.

Screening for polymorphisms in the analyzed fragment was conducted using the SSCP (Single Strand Conformational Polymorphism) technique. 4 µL of the PCR product were mixed with 8 µL of buffer (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, and 0.025% xylene cyanol), denatured (98°C/10 min) and rapidly chilled on ice. Electrophoresis conditions were: 12% polyacrylamide gel (acrylamide : bisacrylamide 29:1, 1 TBE), 150 V/30 min followed by 120 V 16 h. After 16 h of electrophoresis, the polyacrylamide gel was silver-stained using the standard protocol. One PCR product from each SSCP pattern was sequenced by Sirui-Jin Company in order to specifically recognize the polymorphism type and location.

Statistical analysis

Allelic frequencies, genotype frequencies, Hardy-Weinberg equilibrium, gene homozygosity, polymorphism information content (PIC) and effective allele numbers were statistically analyzed using the POPGENE Version 1.31 software. Associations between genotypes of SNP1 of RETN gene and five meat quality indexes (BF, LMA, LMD, MAR and IMF) were analyzed using General Linear Model (GLM) procedure in SPSS software (version 17.0). The following models were used:

$$Y_{ijkl} = \mu + Gi + S_j + BF_k + Ma_l + e_{ijkl}$$

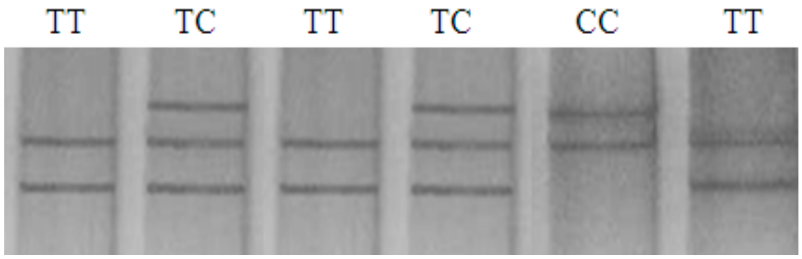

Figure 1. The SST PCR-SSCP defined from genomic DNA amplifications in a silver-stained gel. The observed SSCP alleles are indicated at bottom.

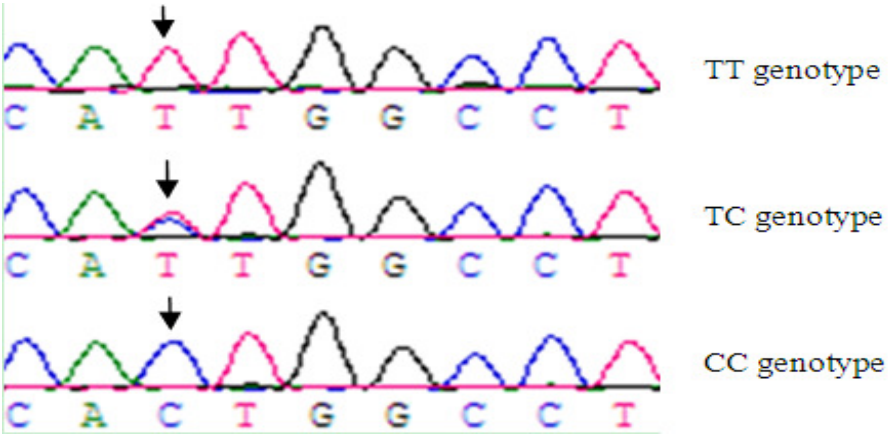

Figure 2. DNA sequencing tracing of exon 2 and its flanking region at the bovine RETN locus.

Where, Y_{ijkl} is the meat quality traits measured on bovine, μ is the overall population mean, Gi is the genotypic effect (TT, TC and CC), S_j is the fixed effects of Sex, BF_k is the fixed effect of the breed and farm; Ma_l stands for the regression variable for age and finally, while e_{ijkl} is the random effect of the environment.

RESULTS AND DISCUSSION

Screening for polymorphisms in all the analyzed fragments revealed the presence of one SNP in the RETN gene (Figure 1). In order to clearly understand the detailed genetic variation within the bovine RETN gene, the polymorphic DNA amplification fragments between exon 2 and its flanking region were sequenced, and the sequence data corresponds to the polymorphic patterns as shown in Figure 2. The polymorphism found in the RETN gene turned out to be a novel one. There have been no studies on its association with meat quality traits of *B. taurus* until now. The tested SNP, g.2528T>C (RETN), alter the amino acid sequence. It was a missense mutation in exon 2 of the RETN gene causing substitution of isoleucine by threonine. The two amino acids are all very important essential amino acid in animals belonging to the group of aliphatic amino acids, but have different polarity – polar for threonine and non-polar for isoleucine acid because of their different side chain charge. Thus the different side chain of the two amino acids may potentially affect the protein shape. In addition, there is a significant difference between isoleucine and threonine hydropathy index: threonine is hydrophilic while isoleucine is hydrophobic.

Table 1 and 2 shows the values of population genetic indexes in all the breeds used in this study. The values of gene heterozygosity, and PIC varied from 0.1068 and 0.1011 to 0.4863 and 0.3681, respectively, for all the six breeds studied. Whereas, gene homozygosity and effective allele numbers of the bovine RETN locus in six breeds of the *B. taurus* varied from 0.5137 to 0.8932 and 1.1196 to 1.9468, respectively. Gene homozygosity is higher than gene heterozygosity in all studied cattle breeds. In almost all breeds (except for Luxi and Luxi × Simmental) the SNP at the RETN gene was polymorphic. The values of PIC for He of QC and NY breed in the loci were higher than that of other populations, which implied that the polymorphism and genetic variation of these two breeds were higher than that of other populations. Taking into consideration the distribution patterns, the polymorphism in two breeds (QC24 and NY24) were selected for further studies on association with meat quality traits.

Table 1. Genotype frequencies (%) of the RETN gene for the SNP in cattle populations.

Breed	Gene (SNP genotype and amino acid substitution) (g. 2528T > C, I47T)			Total	Allelic frequency		χ^2 (HW)
	TT	TC	CC		T	C	
Qinchuan	0.4986 (184)	0.1680 (62)	0.3334 (123)	369	0.5827	0.4173	158.07
Luxi	0.7813 (50)	0.0000 (0)	0.2187 (14)	64	0.7813	0.2187	64.00
Nanyang	0.3836 (28)	0.4932 (36)	0.1233 (9)	73	0.6301	0.3699	0.2453
Jiaxian	0.7083 (34)	0.1875 (9)	0.1042 (5)	68	0.8021	0.1979	8.05
Xia'nan	0.3433 (23)	0.5821 (39)	0.0746 (5)	67	0.6343	0.3657	4.35
Luxi × Simmental	0.9434 (50)	0.0000 (0)	0.0566 (3)	53	0.9434	0.0556	36.80

HW = Hardy-Weinberg equilibrium; $\chi^2_{0.01} = 9.210$, $\chi^2_{0.05} = 5.991$

Table 2. Population genetic indices at the RETN locus in cattle populations.

Breed	Gene homozygosity	Gene heterozygosity	Effective allele number	PIC
Qinchuan	0.5137	0.4863	1.9468	0.3681
Luxi	0.6582	0.3418	1.5193	0.2834
Nanyang	0.5339	0.4661	1.8731	0.3575
Jiaxian	0.6825	0.3175	1.4652	0.2671
Xia'nan	0.5361	0.4639	1.8654	0.3563
Luxi×Simmental	0.8932	0.1068	1.1196	0.1011

PIC = Polymorphism information content.

Table 3. Association between the RETN g.2528T > C polymorphism with selected meat quality traits.

Trait	Breed	RETN (g.2528 T > C) (mean ± SE)			P-value
		TT	TC	CC	
MAR	QC (n = 269)	3.76 ± 1.000	3.25 ± 0.715^{ab}	3.17 ± 0.134^{b}	0.012
	NY (n = 73)	3.30 ± 0.180^{a}	2.77 ± 0.192	2.25 ± 0.185^{b}	0.037
IMF	QC (n = 269)	8.03 ± 0.156^{a}	7.71 ± 0.297^{b}	7.47 ± 0.157	0.024
	NY (n = 73)	-	-	-	-

^{a,b}Means with different superscript lower case letters are significantly different ($p < 0.05$). MAR, Ultrasound marbling score; IMF, intramuscular fat content.

In this study, we also analyzed the relationship between the SNP, namely g.2528T>C, a missense polymorphism in the RETN gene and meat quality traits in QC and NY *B. taurus* at 24 months (QC24, NY) and in QC *B. taurus* at 18 months (QC18). Statistical analysis of an association between the tested polymorphism and selected meat quality traits revealed a significant relationship (Table 3). The g.2528T>C polymorphism was associated with marbling score and intramuscular fat content in QC24 and intramuscular fat content in NY, while non significant associations were observed in QC cattle of 18 months old (data not shown).

Furthermore, our results suggested the TT genotype was associated with a relatively higher marbling score (3.76 ± 0.1000/3.30 ± 0.180) when compared with the TC (3.25 ± 0.134) and CC (2.25 ± 0.185/3.30 ± 0.180) in QC and NY breeds. In QC breed, we also found that TT genotype was associated with an increased intramuscular fat content by (8.03 ± 0.156), when compared with the TC (8.03 ± 0.156). No associations were found in case of the other meat quality traits (backfat thickness, loin-muscle area and loin-muscle depth) in QC24 and NY24.

Until now, several association studies between the RETN and quantitative traits were carried out in humans and pigs (Pan et al., 2008; Jakub et al., 2009; Takayoshi et al., 2009). It should also be mentioned that the porcine RETN gene, mapped to SSC2q21 within the QTL region for several meat quality traits (e.g., backfat thickness and fat to meat ratio), is considered as a positional candidate for fat deposition in pigs (Cepica et al., 2002; Lee et al., 2003). In the present study, we found a significant

association between the missense substitution SNP: g.2528T>C (RETN) polymorphism and marbling score and intramuscular fat content in QC24 cattle (n = 253) and marbling score in NY24 (n = 73). So, we hypothese that the SNP: g.2528T>C of the RETN gene found in our study, may be a positional candidate for meat quality traits in *B. taurus*. Interestingly, the missense substitution we found had a similar effect on marbling score index simultaneously in QC and NY two different cattle breeds. And we know that, marbling score is a very important meat quality index that is mostly used by beef industry and beef market to measure the beef quality and the grade.

Conclusion

Our study on polymorphisms of the RETN gene showed that one polymorphism in the RETN gene is potentially associated with *B. taurus* meat quality traits. Thus further studies are recommended.

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REFERENCES

Boden G (1997). Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes, 46: 1–10.

Bouchard L, Weisnagel SJ, Engert JC (2004). Human resistin gene polymorphism is associated with visceral obesity and fasting and oral glucose stimulated C-peptide in the Quebec Family. Study J. Endocrinol. Invest. 27: 1003–1009.

Cepica S, Rohrer GA, Masopust M, Kubickova S (2002). Partial cloning, cytogenetic and linkage mapping of the porcine resistin (RETN) gene. Anim. Genet. 33(5): 381–383.

Holcomb IN, Kabakoff RC, Chan B, Baker TW (2000). FIZZ1, a novel cysteinerich secreted protein associated with pulmonary inflammation, defines a new gene family. Embo. J. 19: 4046–4055.

Jakub C, Joanna NW, Magdalena B (2009). Association studies on the porcine RETN, UCP1, UCP3 and ADRB3 genes polymorphism with fatness traits. Meat Sci. 83: 551–554.

Keller U (2006). From obesity to diabetes. Int. J. Vitamin Nutr. Res. 76: 172–177.

Kim KH, Lee K, Moon YS, Sul HS (2001). A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J. Biol. Chem. 276: 112–126.

Lee SS, Chen Y, Moran C, Cepica S (2003). Linkage and QTL mapping for Sus scrofa chromosome 2. J. Anim. Breed. Genet. 120(31): 138–143.

Michael WR, Silvana O, Philipp ES, Luciano R (2003). Adipose-derived resistin and gut-derived resistin-like molecule-b selectively impair insulin action on glucose production. J. Clin. Invest. pp. 111–225.

Moon B, Kwan JJ, Duddy N, Sweeney G (2003). Resistin inhibits glucose uptake in L6 cells independently of changes in insulin signaling and GLUT4 translocation. Am. J. Physiol. Endocrinol. Metab. 285: E106–115.

Mullenbach R, Lagoda PJ, Welter C,(1989). An efficient salt-chloroform extraction of DNA from blood and tissues. Trends Genet. 5: p. 391.

Osawa HK, Yamada H, Onuma, Onuma H, Onuma, Murakami AOch M, Kawata K, Nishimiya T, Niiya T,Shimizu Nishida W, Hashiramoto M, Kanatsuka A,Fujii Y, Ohashi J, Makino H (2004). The G/G genotype of a resistin single-nucleotide polymorphism at 420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/ 3, Am. J. Hum. Genet. 75: 678–686.

Osawa HW, Nishida Y, Tabara, Kawamoto R, Ohashi J, Ochi M, Onuma H, Nishida W, Yamada K, Nakura J, Kohara K, Miki T, Makino H (2007). Plasma resistin, associated with single nucleotide polymorphism 420 is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population, Diabete Care, 30: 1501–1506.

Pistilli EE, Gordish-Dressman H, Seip RL, Devaney JM, Thompson PD, Price TB, Angelopoulos TJ, Clarkson PM, Moyna NM, Pescatello LS, Visich PS, Zoeller RF, Hoffman EP, Gordon PM (2007). Resistin polymorphisms are associated with muscle, bone and fat phenotype in white men and women. Obesity, 15: 392–402.

Satoh H, Nguyen MT, Miles PD, Imamura T, Usui I, Olefsky JM (2004). Adenovirus- mediated chronic hyper-resistinemia leads to *in vivo* insulin resistance in normal rats. J. Clin. Invest. 114: 224–231.

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA (2001a). The hormone resistin links obesity to diabetes. Nature, 409: 307–312.

Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, Enders GH, Silberg DG, Wen X, Wu GD, Lazar MA (2001b). A family of tissue-specific resistinlike molecules. Proc. Natl. Acad. Sci. USA. 98: 502–506.

Takayoshi T, Eitaro N, Atsuko W, Yoji H, Keikjo N, Hideki KN, Nobuhisa K, Norihiro H, Nobuyuki S, Yoshitaka N, Toshimitsu T, Makoto O, Yutaka J (2009). Polymorphism in resistin promoter region at S420 determines the serum resistin levels and may be a risk marker of stroke in Japanese type 2 diabetic patients. Diabetes Res. Clin. Practice, 84: 179–186.

Van den Maagdenberg K, Stinckens A, Claeys E, Buys N (2008). Meat Sci. 80(2): 293–303.

Wolf G (2004). Insulin resistance and obesity resistin a hormone secreted by adipose tissue. Nutr. Rev., 62: 389–394.

Ying-bin PAN, Yan-fen LIU, You LIU, Zhi-jie GAO (2008). SNP Analysis of Resistin Gene in Six Pig Breeds by PCR-SSCP. J. Agric. Biotechnol. 16(3): 402–406.