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Full Length Research Paper

The peroxisome proliferators-activated receptor gamma (PPARG) gene polymorphisms and associations with body measurements of cattle

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Peroxisome proliferators-activated receptor gamma (PPARG) is an important regulator in the regulation of adipose differentiation and development. The mutations of the PPARG in human had been shown to be associated with type II diabetes, fat distribution and body weight. The functional importance of the PPARG makes it a good candidate to search molecular markers in marker assistant selection in cattle breeding. All the mRNA region of the PPARG gene within 760 individuals of four Chinese cattle breeds was scanned and four single nucleotide polymorphisms (SNPs) (-110G>C, -27C>T, +20A>G, +1344G>T) of the PPARG were detected in three Chinese indigenous cattle breeds (Qinchuan, Nanyang and Jiaxian cattle), rather than Chinese Holstein cattle. The mutations -110G>C, -27C>T and +20A>G located in the Exon1 of the PPARG and were under linkage disequilibrium. The individuals with these three mutations had smaller body measurements. This information could help animal scientists to develop genetic markers or biomarkers to assist with beef production.

Key words: Peroxisome proliferators-activated receptor gamma (PPARG) gene, polymorphisms, cattle, association analysis.

INTRODUCTION

Adipose tissue is a cell mass contributing not only to the storage of fat, but a sophisticated ensemble of cellular components with highly specialized and complex functions. In addition to managing the most important energy reserve of the body, it secretes a multitude of soluble proteins called adipokines, which have different effects on the homeostasis of the whole body. The expression of these adipokines is an integrated response to various

signals received from many organs, which depends heavily on the integrity and physiological status of the adipose tissue. One of the main regulators of gene expression in fat is the transcription factor peroxisome proliferators-activated receptor gamma (PPARG), which is a fatty acid- and eicosanoid-dependent nuclear receptor that plays key roles in the development and maintenance of the adipose tissue (Anghel and Wahli, 2007). Furthermore, synthetic PPARG agonists are therapeutic agents used in the treatment of type II diabetes (Moller, 2001). Evidence from studies of humans and mice has indicated PPARG to be not only a key factor for adipogenesis but also a critical determinant of body fat distribution (Tsai and Maeda, 2005). Association analysis of the PPARG Pro12Ala variants reveals opposing associations with body weight (Deeb et al., 1998; Ek et al., 1999; Doney et al., 2002). The functional importance of PPARG makes it a good candidate to search molecular markers in Marker Assistant Selection (MAS) in cattle breeding. The study would search the mutations of PPARG in several Chinese indigenous cattle breeds, and analyse the associations between the mutations

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Abbreviations: PPARG, Peroxisome proliferators-activated receptor gamma; MAS, Marker assistant selection; NY, Nanyang; QC, Qinchuan; JX, Jiaxian; CH, Chinese Holstein; PCR-SSCP, polymerase chain reaction single-strand conformation polymorphism; SNPs, single nucleotide polymorphisms; RFLP, restriction fragment length polymorphism; LD, Linkage disequilibrium; BMI, body mass index.

Table 1. Primers for Exon1 to Exon7 of *PPARG* gene in cattle.

Loci	Sequence	Tm (°C)	Length (bp)
Exon1	5' CAGCCCAATCTGGAGGTGAC 3'	64	362
	5' CAGTGAACAGCCTTATGACGC 3'		
Exon2	5' CTGGGAGCATTACCCTACT 3'	62	350
	5' CTCAGGGCTAACGTCACAG 3'		
Exon3	5' TTTTCCTGTGATGATTGTCTG 3'	63	281
	5' CAACACGAAATGAAAACCTA 3'		
Exon4	5' TCCCTCGCCCATATTCCT 3'	56	213
	5' TAGCAGCCGTGATGAGAAG 3'		
Exon5	5' CTCATTCATCCTGCCCTTTC 3'	56	261
	5' CCCCAAATGAAGACAGTAGAAG 3'		
Exon6	5' TTCCAACCTCAGCCACTTTC 3'	56	503
	5' CCAGCAGCAGCTTACCTC 3'		
Exon7	5' TATGCCTCAGATGTGGGAC 3'	57	579
	5' GAGAATGGGGTGGGGAAC 3'		

and the body measurements of cattle.

MATERIALS AND METHODS

Animals and management

Blood samples were obtained from 760 individuals of four Chinese cattle breeds: Nanyang (NY, n = 287), Qinchuan (QC, n = 272), Jiaxian (JX, n = 141), Chinese Holstein (CH, n = 60). The test population of NY and QC cattle were reared in the Henan Nanyang Cattle Conservation Farm and Shaanxi Qinchuan Cattle Conservation Farm of China, respectively. The cattle in the conservation farm were under the same nutrition conditions. The test population of JX cattle were dispersed in the village in Henan province of China. The body measurements (including body height, body length and heart girth) of 95 NY cattle were collected at 6, 12, 18 and 24 months, respectively. These body measurements of 112 JX cattle and 84 QC cattle were collected too, which were all more than two years old. Several milk traits (milk fat rate, milk protein rate and 305 ds milk yield) of the 60 CH cattle were collected in the diary cattle farm.

Single nucleotide polymorphisms (SNPs) identification and genotyping

DNA samples were extracted from blood samples firstly and then polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) (Zhang et al., 2007) was used to scan the mutant of the *PPARG*. The primers for the seven Exons of *PPARG* were designed individually (Table 1).

The PCR products with different PCR-SSCP patterns were sequenced to find the mutations within the *PPARG* and then, PCR-restriction fragment length polymorphism (RFLP) (Michael et al., 1998) was used to investigate the genotypes of the four SNPs (-110G>C, -27C>T, +20A>G, +1344G>T) individually. Mutation -110G>C, -27C>T and +20A>G was located in Exon1. The primers for this region were Forward: 5' AAACGGACGTCTTGGCTCATT 3'; Reverse: 5' CTCTCTGGGTCAATAAGAGGA 3'. Mutations -110G>C and -27C>T can be detected by restriction enzyme *BanI* and *HinfI*, respectively. A mutation was induced to the forward primer to remove a *HinfI* restriction enzyme site, which is no polymorphism in

the test breeds, for a clear background of the *HinfI* electrophoresis pattern. Another mutation was induced to the reversed primer to add a *Sau96I* restriction enzyme site, so that forced PCR-RFLP can be used to investigate the genotype of mutation +20A>G. Mutation +1344G>T located in the Exon7 of *PPARG*, could be detected by restriction enzyme *PstI* naturally. The allele assigned of the four mutations is presented in Table 2.

Statistical analysis

Linkage disequilibrium of the SNPs and haplotype frequencies were estimated through PowerMarker (Liu and Muse, 2005). The general linear model (GLM) in the Statistical Package for Social Sciences (SPSS) (version 13.0) was used to evaluate the association between the genotypes and haplotypes of *PPARG* and the body measurements of cattle. Independent variables were *PPARG* genotype (or haplotype), breed, age and sex. The GLM was displayed as followed:

$$Y_{ijklm} = u + G_i + B_j + A_k + S_l + e_{ijklm}$$

Where, Y_{ijklm} , Observed value; u , overall mean for each trait; G_i , fixed effect associated with i_{th} genotype; B_j , fixed effect associated with j_{th} breed; A_k , fixed effect associated with k_{th} age; S_l , fixed effect associated l_{th} sex; e_{ijklm} , random error.

The least-squares means (LSM) of the GLM were used to determine the level of significance in pairwise comparisons between genotypes classes. LSM also known as population marginal means, are the values for class means after adjustment for all covariates in the model (Al-Shali et al., 2004). Pairwise comparisons of LSMs were performed using Fisher's protected least significant difference (LSD) t-test procedure.

RESULTS

SNPs genotyping

The results of PCR-SSCP analysis showed that just the Exon 1 and 7 of *PPARG* displayed different electrophoresis patterns (Figure 1). The PCR products of the individuals

Table 2. Allele assign of the SNPs of *PPARG* gene.

SNPs	Sequence	Amino acid	Enzyme	Cleavage site
-110G>C	Wild ggtgcg	-	<i>BanI</i>	No
	Mutant ggtgcc		GGYRCC	Yes
-27C>T	Wild gagtc	-	<i>HinfI</i>	Yes
	Mutant gagtt		GANTC	No
+20A>G	Wild gatg/cc	Asp7Gly	<i>Sau96I</i>	No
	Mutant ggtg/cc		GGNCC	Yes
+1344G>T	Wild ctgcag	Gln448His	<i>PstI</i>	Yes
	Mutant ctgcat		CTGCAG	No

The bold letters showed the mutations which remove or add a specific enzyme cleavage site. The letters in the frame showed an artificial mutation was induced into the sequence to form an enzyme cleavage site. For example, **g/c** means that after the "g" was forcibly changed into "c", the mutation +20A>G could be detected by *Sau96I*.

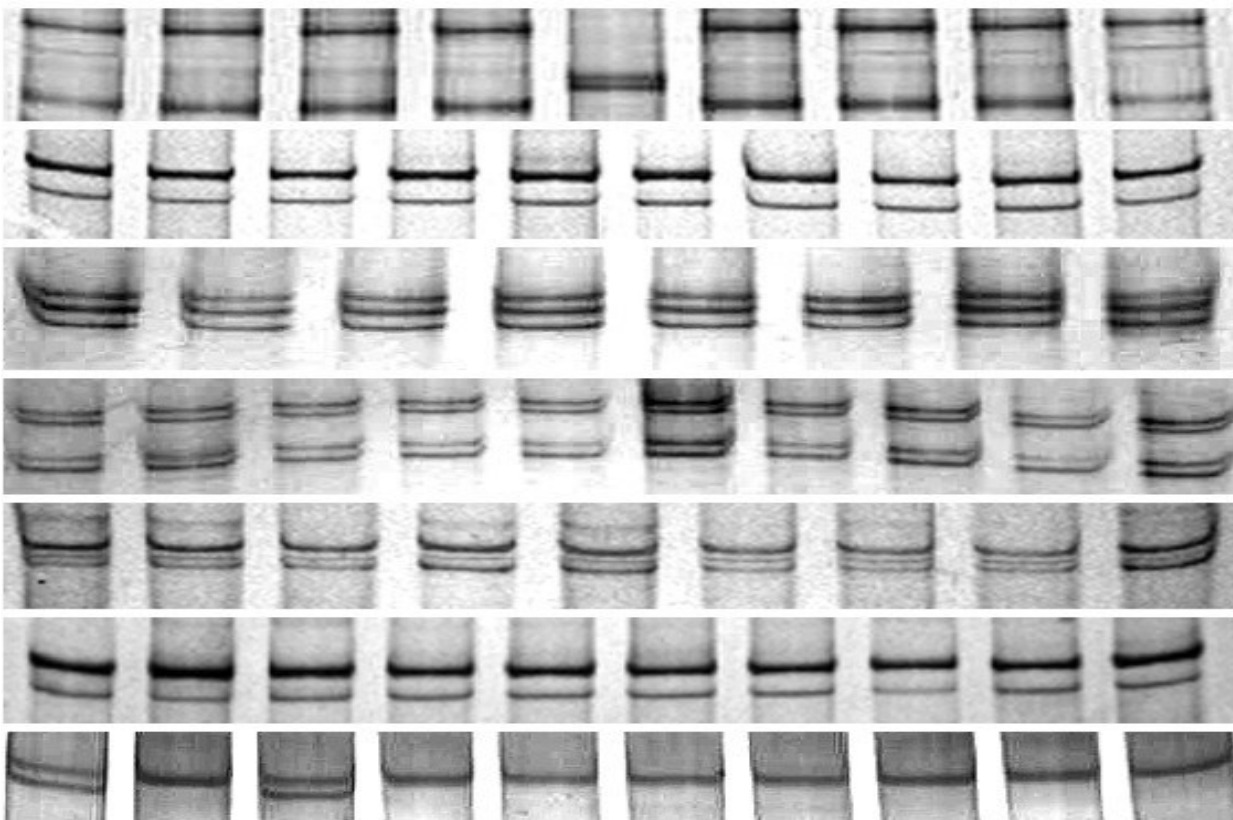


Figure 1. PCR-SSCP patterns of the Exon1 to Exon7 of *PPARG* gene in cattle. The Exon1 to Exon7 was showed from the top to the bottom. The length of the PCR product from Exon1 to Exon7 was 362, 350, 281, 213, 261, 503 and 579 bp, respectively. The electrophoresis voltage was 200 V. The electrophoresis time varied from 2.0 to 3.0 h depending on the length.

with different electrophoresis pattern were sequenced. Four mutations (-110G>C, -27C>T, +20A>G and +1344G>T) in the Exonic region of *PPARG* were detected in the test breeds. Mutations -110G>C and -27C>T located in the 5' UTR, add or remove a restriction enzyme site of *BanI* or *HinfI*, respectively. Mutation +20A>G located in the

Exon1, caused the 7th amino acid changing from Asp to Gly (Asp7Gly). By introducing a mutation to the primer, the mutation Asp7Gly could be genotyped by restriction enzyme *Sau96I*. Mutation +1344G>T located in the Exon7 of *PPARG*, which caused the 448th amino acid changing from Gln to His (Gln448His). The mutation

Table 3. Genotype and allele frequencies of the SNPs of *PPARG* gene.

Parameter	Loci	QC	NY	JX	CH
Genotype frequency	-110G>C (N _{GG} /N _{GC} /N _{CC} /N _T) (P _{GG} /P _{GC} /P _{CC})	266/3/0/269 0.99/0.01/0	225/27/1/253 0.89/0.10/0.01	132/4/0/136 0.97/0.03/0	55/0/0/55 1.00/0/0
	-27C>T (N _{CC} /N _{CT} /N _{TT} /N _T) (P _{CC} /P _{CT} /P _{TT})	238/31/0/269 0.88/0.12/0	206/44/3/253 0.82/0.17/0.01	109/24/3/136 0.80/0.18/0.02	55/0/0/55 1.00/0/0
	+20A>G (N _{AA} /N _{AG} /N _{GG} /N _T) (P _{AA} /P _{AG} /P _{GG})	251/18/0/269 0.93/0.07/0	220/32/1/253 0.87/0.12/0.01	123/12/1/136 0.90/0.09/0.01	55/0/0/55 1.00/0/0
	+1344G>T (N _{GG} /N _{GT} /N _{TT} /N _T) (P _{GG} /P _{GT} /P _{TT})	184/80/9/273 0.68/0.29/0.03	230/35/2/267 0.86/0.13/0.01	102/38/0/140 0.73/0.27/0	50/0/0/50 1.00/0/0
Allele frequency	-110G>C (P _G /P _C /HWE test ^a)	0.99/0.01/1.00	0.94/0.06/0.56	0.99/0.01/1.00	1.00/0
	-27C>T (P _C /P _T /HWE test ^a)	0.94/0.06/0.58	0.90/0.10/1.00	0.89/0.11/0.06	1.00/0
	+20A>G (P _A /P _G /HWE test ^a)	0.97/0.03/0.27	0.93/0.07/0.61	0.95/0.05/0.04	1.00/0
	+1344G>T (P _G /P _T /HWE test ^a)	0.82/0.18/0.68	0.93/0.07/0.64	0.86/0.14/0.13	1.00/0

^a The p-value greater than 0.05 means the locus was under Hardy-Weinberg equilibrium in the test population.

Table 4. Multi-locus linkage disequilibrium analyses of the SNPs of *PPARG* gene.

Loci	QC ^a	NY	JX
-110G>C - -27C>T - +20A>G	< 0.01	< 0.01	< 0.01
+20A>G - +1344G>T	0.78	1.00	0.06

^a The p-value smaller than 0.05 means these loci were under linkage disequilibrium.

Gln448His could be genotyped by restriction enzyme *Pst*I naturally. The genotype and allele frequencies of the four mutations are shown in Table 3. No mutations were detected in the CH breed, except the mutation +20A>G in the JX breed, all mutations in the test breeds were under Hardy Weinberg equilibrium.

Linkage disequilibrium (LD) and haplotype analysis

Multi-locus linkage disequilibrium test of the four SNPs are shown in Table 4. The mutations -110G>C, -27C>T and +20A>G were under linkage disequilibrium in QC, NY and JX breeds, but the mutation +1344G>T was not linked with this haplotype block.

Association analysis

The associations between the four SNPs and the cattle

body measurements were analyzed individually (Table 5). The individuals with the mutation -110G>C had smaller body length and heart girth ($P < 0.05$), whereas with the mutations, -27C>T and +20A>G had smaller body height ($P < 0.05$). No association was observed between the mutation +1344G>T and the body measurements of cattle.

The difference between the wild haplotype GG-CC-AA and the mutant haplotype GC-CT-AG is shown in Table 6. The individuals with haplotype GC-CT-AG had smaller body length and heart girth ($P < 0.05$), which also means the mutations -110G>C, -27C>T and +20A>G had a negative effects on the body measurements of cattle.

DISCUSSION

All three types of peroxisome proliferator-activated receptor (PPARG, PPARA and PPARD) are important

Table 5. Associations between the four SNPs and the body measurements of cattle.

Body measurement	-110G>C			-27C>T			+20A>G			+1344G>T		
	GT	LSM	SE	GT	LSM ^a	SE	GT	LSM	SE	GT	LSM	SE
Body height	GG	121.74	0.24	CC	121.80 ^a	0.25	AA	121.76 ^a	0.25	GG	121.64	0.28
	GC	120.09	0.88	CT	121.93 ^a	0.67	AG	122.00 ^a	0.71	GT	121.95	0.53
	- ^b	-	-	TT	116.50 ^b	2.23	GG	112.50 ^b	3.89	TT	122.36	1.53
Body length	GG	134.42 ^a	0.40	CC	134.7	0.41	AA	134.48	0.41	GG	134.37	0.45
	GC	131.24 ^b	1.46	CT	132.95	1.11	AG	133.87	1.19	GT	134.02	0.88
	-	-	-	TT	139.67	3.7	GG	139	6.5	TT	129.94	2.52
Heart girth	GG	165.04 ^a	0.45	CC	165.12	0.47	AA	164.99	0.47	GG	165	0.51
	GC	160.74 ^b	1.66	CT	165.24	1.27	AG	165.56	1.35	GT	164.79	0.98
	-	-	-	TT	168	4.23	GG	168	7.34	TT	162.67	2.82

^ap Values of non-random difference between LSMs in pairwise comparisons were estimated in the general linear models procedure. Within a column, LSMs without a common superscript differ ($p < 0.05$); ^b'-' in the table means the genotype was not detected in the test breed.

Table 6. Associations between the haplotypes of *PPARG* gene and the body measurements of cattle.

Body measurement	Haplotypes	LSM ^a	SE	P-value
Body height	GG-CC-AA	121.80	0.25	0.06
	GC-CT-AG	120.09	0.87	
Body length	GG-CC-AA	134.70 ^a	0.41	0.02
	GC-CT-AG	131.24 ^b	1.42	
Heart girth	GG-CC-AA	165.12 ^a	0.47	0.01
	GC-CT-AG	160.74 ^b	1.65	

^ap Values of non-random difference between LSMs in pairwise comparisons were estimated in the general linear models procedure. Within a column, LSMs without a common superscript differ ($p < 0.05$).

regulators of lipid metabolism. Although, they share significant structural similarity, the biological effects associated with each PPAR isotype are distinct. For example, PPARG controls lipid storage and adipogenesis. The different functions of PPARs *in vivo* can be explained as part by the different N terminus structure (Hummasi and Tontonoz, 2006). This implies that the mutations located at the 5' of the PPARs potentially had important influence on the phenotype. The validated function of Pro12Ala existing in Exon1 of human *PPARG* also proved the functional importance of this locus (Deeb et al., 1998; Ek et al., 1999; Doney et al., 2002). The mutations -110G>C, -27C>T and +20A>G existed in the same region of *PPARG* in cattle, which means that these variations potentially have important regulatory roles in the body development. The negative effects to the body measurements of these mutations were similar with the mutation Pro12Ala in human, which is also associated with lower body weight (Doney et al., 2002). But the mechanism of how these mutations affect the body

development is unknown yet. The Mutation +1344G>T did not show any influence in the body measurements in the test breeds though it caused an amino acid change (Gln448His). Different situation is in human where the mutation 1431C>T was consistently associated with higher body mass index (BMI) and also linked with the mutation Pro12Ala (Doney et al., 2002).

Since the *PPARG* is crucial in adipose differentiation, it is expected that the mutations in the *PPARG* could affect the milk traits. But four of the mutations were just detected in the three Chinese indigenous breeds (QC, NY, JX), rather than CH. A possible explanation is the negative selection against these mutations. QC, NY and JX are all indigenous Chinese cattle breeds. They were not as highly selected as CH, which was selected just for milk production for a long time. Another explanation is breed difference, for example, we also could not identify the mutation Ala18Val in test Chinese cattle breeds, which was reported in Japanese Black cattle (Katsuhiro et al., 1999).

The increased amount of the molecular markers asso-

ciated with cattle performance were reported (Ge et al., 2003; Schenkel et al., 2005; Zhang et al., 2008), but before using these mutations in a marker assisted selection program, this association should be validated in the target population. The cattle performances are complicated traits which are regulated by lots of factors, so the associations between the molecular markers and the performance can be different in different populations. Some markers regulate the body development through the linkage with some unknown functional mutations and this association could be lost due to recombination.

Four mutations were identified in the *PPARG* gene of Chinese cattle. Three of which under linkage equilibrium were associated with lower body measurements. This information could help animal scientists to develop genetic markers or biomarkers in cattle breeding, individuals with these mutations should be eliminated through selection. According to the biological function of the *PPARG*, it is worthy to investigate the associations between these mutations and the meat quality traits in the next study.

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