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Full Length Article

Correlation between Uncoupling Protein3 (UCP3) Gene Polymorphism and Growth Traits of Hybrid Simmental

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Abstract

The uncoupling protein3 (UCP3) influences energy balance, basic metabolism and lipid metabolism. To study the relationship between uncoupling protein3 (UCP3) gene and growth traits in hybrid cattle (Simmental×Native Yellow Cattle), five novel SNPs (g.5465 G→A, g.7921 C→G, g.7953 A→G, g.8018 T→C, g.8162 T→C) were identified by PCR-SSCP and sequencing. Result shown that g.5465G→A, g.8162T→C had moderate genetic diversity (0.250<PIC<0.500). Correlative analysis of five SNPs and body traits showed that SNP1 and SNP5 were associated with body weight, body length, body height, chest circumference ($P < 0.05$ or $P<0.01$) in hybrid cattle aged birth month, weaning months, six, twelve and eighteen months. The polymorphism, generated by T8162C mutation had the highest body height, body length of the hybrid cattle aged from birth month to eighteen months ($P<0.01$). Combination genotypes GACC had better performance than GGCC. Results showed that the polymorphism in bovine UCP3 might be one of the significant genetic factors that affect body performance, meanwhile, which supplies a good foundation and useful information for further study on UCP3 gene and cattle breeding.

Keywords: UCP3; Hybrid cattle; Polymorphism; Growth trait

Introduction

The uncoupling protein (UCP) is a specific protein functioning as a vector of transshipment proton controlling oxidative phosphorylation, thermogenesis or producing energy (Brondani *et al.*, 2014). It is well known that UCP can cause obesity (Boldman *et al.*, 1995; Chen *et al.*, 2012). Up to date, six members were found, they were UCP1, UCP2, UCP3, UCP4, UCP5 (or BMCP1) and UCP6 (Qiao, 2007). The UCP3 played a key role in balance controlled of energy, and also was necessary for lipid and basic metabolism (Damon *et al.*, 2000; Fallin *et al.*, 2001; Ricquier and Bouillaud, 2003). For human, C>T mutation of UCP3 influences energy-metabolism (Cieslak *et al.*, 2009).

The UCP3 gene of bovine is located on chromosome 15 that includes 6exons and 5introns (Henderson, 1986). UCP3 gene contains six functional domains and a combination zone of purine nucleotide (Hickford *et al.*, 2009). Scholars revealed that obesity was related to mutations in the UCP3 (Gura, 1998; Knoll *et al.*, 2000; Huang, 2010). Numerous studies have pointed out mutation of UCPs gene was associated with lipid metabolism and obesity in human (Lapice *et al.*, 2014). For chicken, two SNPs were found in UCP gene that it was associated with carcass weight and seven-week weight (Leng, 2012). For pigs, UCP3 gene may be used as a genetic marker for carcass traits for marker-assisted selection (Wu *et al.*, 2007). 5′-UTR of UCP3 gene subsequently digested with restriction enzyme, which had significant influence in muscle and fat transformation of energy (Li *et al.*, 2006). Association between intron2 in UCP3 gene with live weight, slaughter weight was confirmed by Sherman, who found that one SNP at intron3 and association with gaining weight, growth traits and feed conversion rate (FCR) (Mexitalia *et al.*, 2013). Different genotypes in 3′UTR of UCP3 gene were associated with body length, backfat thickness, loin eye area (Mostyn *et al.*, 2004). The Thr842Met was detected in ORF of UCP3 that influenced meat traits (Nakayma *et al.*, 2014). The missense mutation T946C had significant associated with backfat thickness (Liu *et al.*, 1998). Furthermore, one mutation in intron4 of UCP3 gene was found that affected carcass, meat traits (Sherman *et al.*, 2008). For cattle, The G→A polymorphisms was detected in intron3 of UCP3 gene which related to ADG (Stone *et al.*, 1999). Three SNPs

were found in Qinchuan cattle and were related to carcass, meat traits called AA genotype (Suboyama-Kasaoka *et al.*, 1998). B allele was advantageous among three cattle breeds (Tu *et al.*, 2004). Moreover, polymorphisms of UCP3 gene was in association with growth traits in Nanyang cattle (Urhanmaer *et al.*, 1998). Studies shown homozygote individuals had better performance in UCP3-Bgl I locus which had significant influence in body weight(BW), body height(BH), body length(BL) at 6 months and in BH, BL, chest girth(ChG) in 24 months (Vidal-Puig *et al.*, 1997). Other studies on UCP3-BglIlocus also showed the association with 15 months ChG, BL, BH and 12 months BH, 8 months BL. Cattle with AA genotype were greater than other individuals ($P<0.05$) (Werner *et al.*, 1999).

Ningxia, with an area of 66,400 square kilometers, is China's smallest province, accounting for only 0.69%, however, the number of beef cattle groups account for 2.6% of the country. In northwest of China, Hybrid cattle is large groups as beef cattle, especially in Ningxia Hui Autonomous Region. To detect SNPs in UCP3 gene among these superior hybrid cattle and analyze the association between genotype and growth performance was the purpose of this study. We hope the results can be used as genetic markers in Ningxia beef cattle groups, and supply a good foundation for further study on UCP3 gene and provide some useful information on cattle breeding.

Materials and Methods

Animals

The experimental animals were chosen from the same feedlot (Yinchuan, Ningxia), a total of 243 individuals of Hybrid Simmental. The DNA samples that used for experiment were achieved from blood samples by phenol-chloroform isolation method, soluble in TE solution, saved at -20°C. Statistical analysis used data of recording individuals' BW, BH, BL and ChG for different periods (0, 3, 6, 12 and 18 months). All the experiments were conducted in conformity to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

SNPs Identification and Genotyping

The two pairs of primers (Table 1) used to amplify the coding region of UCP3 gene were got from the Genbank (accession AF092048). PCR reaction mixture was performed a 25 μL system which was involving 1% DEPC water 9.5 μL, TaqPCR MasterMix 12.5 μL, 10 pmol, each primer 1 μL, 50ng genomic DNA 1 μL. The total process included, denatured at 94℃ for 5 min, followed of 94℃ for 35s, annealed for 40s at 53℃, extend at 72℃ 40s and continued 10 min for a final extension. Aliquots of 2 μL PCR products and 8 μL denaturing solution (0.025% xylene-cyanole, 95% formamide, 25 m*M* EDTA and 0.025% bromophenol blue) were mixed, then heated for 10 min at 98℃, and refrigerated on ice.

Table 1: The primers for SCP-SSCP analysis

Loci	Variant location	Primer sequence (5′—3′)	Size(bp)	Tms(℃)
P1	exon3	F: CACCCTCCGATTTACCACA-3′ R: CTCTGCCTCTGAGTCTGC-3′	479	53
P2	exon6	F: GACGGAGCACAAGCACTA-3′ R: AGGGGAGGAAGGGAGACG-3′	399	

At a constant temperature of 4℃, the denatured DNA was subjected to 10% or 12% PAGE in 0.5×TBE buffer and steady voltage (180V) for 5 to 8 h. The gel was stained with 0.1% silver nitrate. The sequences that obtained were edited and arranged by the DNAMAN, also, the blast algorithm was used. (http://www.ncbi.nlm.nih.gov/).

Statistical Analysis

Gene frequency was determined by direct counting. Use the POPGENE software to calculate Hardy-Weinberg equilibrium (HWE), which contained to test likelihood ratio for different locus and the number of observed and effective alleles (Yeh *et al.*, 1999). To calculate Polymorphism information content (PIC) by Botstein's methods (Schumm *et al.*, 1988). The formulas were as followed:

$$PIC = 1 - (\sum_{i=1}^{n} p_i^2) - (\sum_{i-1}^{n-1} \sum_{j-i-1}^{n} 2 p_i^2 p_j^2)$$

Where P_i and P_j is the frequency of the ith and jth allele, respectively, and n is the number of alleles.

Use general linear model (GLM) procedure of SAS software (Version 8.02) to achieve the association between single SNP marker genotype and growth traits through the least squares method. Actually, the effects of age of cattle, season of birth, age of male-female animals, sex, farm and random effects (animal, permanent environment, and residual) should have been considered in the full animal model, but when I chose the animals, already considered all these effects that those did not affect variability of the traits in the experiment groups.

The linear model consists of fixed effects of traits and genotype. The model:

$$Y_{ij} = \mu + np_j + e_{ij}$$

Where y_{ij} is the phenotype of the animal growth performance, μ means the population, np_j is the fixed effect associated with jth genotype, e_{ij} is the standard error. Use the least square means estimates (LSM) with standard errors to calculate correlation of different genotypes and growth traits.

Association between combined genotypes and BW/BH/BL were calculated to research the potential interaction between the different genotypes. The interactions between the different combined genotypes were involved ed as a fixed effect, it also included similarity of single marker association analysis model and the model. The analyses were performed by SAS software.

Results

Identification of SNPs Polymorphism and Genotyping

Firstly, we analyzed polymorphisms by PCR-SSCP fragments, the figure showed two SSCP patterns in P1 and P2 loci (Fig. 1). Five novel SNPs were detected by using sequencing method (AF092048) (Fig. 2). The SNPs were all transition, one of them in P1 locus, named SNP1 (G5465A), others in P2 locus, named SNP2 (C7921G), SNP3 (A7953G), SNP4 (T8018C), SNP5 (T8162C). The mutation in P1, g.5465G→A (SNP1), leads to a substitution of alanine to threonine and others in P2, g.7953 A→G (SNP3), g.8018T→C (SNP4), g.8162T→C (SNP5), leads proline to arginine, cysteine to arginine, tyrosine to histidine, respectively. Interestingly, depending on the lab works and chromatograms sequencing of SNP2 to SNP5, we found novelty issues, when SNP2 to SNP4 were homozygous mutation, SNP5 was homozygous mutation, opposites, when SNP2 to SNP4 were non-mutation, SNP5 was heterozygosis mutation, so SNP2 to SNP5 were in complete linkage disequilibrium. Thus, we only analyzed the genotype results of SNP5, it was equivalent to analyze the genotyping results of SNP2 to SNP5.

Diversity Analyses

The results showed that the frequency of GG genotype was higher than GA in P1, and AA genotype was not found. In P2, the results showed the frequency of CC genotype was higher than TC, and TT genotype was not found. Normally, using PIC to describe the genetic richness and various function of the number of alleles and allele frequencies. The PIC results suggested that SNP1, SNP5 had moderate genetic diversity (0.250<PIC<0.500), which demonstrated that Hybrid Simmental had great genetic potential at these locus (Table 2).

Association Analysis of Single SNP Marker and Combinations

To analyze the relationships between P1 polymorphisms and growth traits (BW, BH, BL and ChG) in Hybrid Simmental aged at 0, 3, 6, 12 and 18 months (Table 3). In P1 locus, G5465A caused polymorphism, and it was associated with BW, BH, BL of Hybrid Simmental aged from 0 to 18 months, significantly. Different genotype had found associated with growth traits in BW, BH, BL, ChG at 0-3 months, but it did not reach a highly level (P>0.05). The cattle which had AA genotype showed extremely higher BW, BH, BL than BB genotype at 6, 12 months old (P<0.05), meanwhile, ChG did not show significant difference (P>0.05). At 18 months, cattle with AA genotype was higher than BB individuals in regard to great performance (P<0.05).

Table 2: Genotypic and allelic frequencies of two loci within UCP3 gene in Hibird Simmental Cattle

Locus	Total	Observed Genotypes		Allele Frequencies		$\chi 2$	PIC
P1	110	GG	0.5473	G	0.7736	20.798	0.3727
	133	GA	0.4526	A	0. 2264		
		AA	0				
P2	191	TC	0.8643	T	0.4321	127.971	0.3704
	30	CC	0.1357	C	0.5679		
		TT	0				

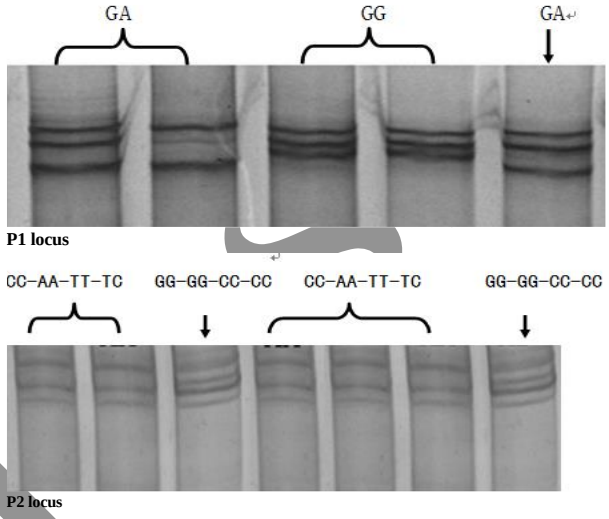


Fig. 1: Different PCR-SSCP patterns of two loci in bovine UCP3 gene on the 10% or 12% PAGE

From Table 4, low polymorphism was found in P2. At 0 months, individuals with AB genotype had higher birth weight (P<0.05). At weaning months and 6 months they did not reach a significant level on different genotype (P>0.05). The cattle with AA genotype displayed particularly higher BW than AB genotype at 12, 18 months (P<0.05); cattle with AA genotype had long BL at birth months (P<0.01), and had greater BL at 3, 6, 12, 18 than AB genotype (P<0.05), AA genotype had lower ChG than AB genotype at 6, 12, 18 month. Individuals with AA genotype and AB genotype had difference in body height values at birth months and weaning months, but it did not show significant difference (P>0.05); AA genotype had grater body height at 6, 12, 18 months (P<0.05). In P2 locus, T8162C mutation caused polymorphism, and it was related to BH and BL of Hybrid Simmental aged at 0 to 18 months, very. The C7921G、A7953G、T8018C mutation could affect early-gained weight and chest girth, but it has no significant effects on growth traits at growth period.

As showed in Table 5, combination genotypes GACC had better BW, BH, BL at 0 to 18 months than GGCC, especially BW of GACC genotypes at 6 to 18 months (P<0.05). The analysis of combination genotype is meaningful than a single SNP with genotype. Another research also proved the inheritance of combinations was more effective than one single SNP (Zhou *et al.*, 2000).

Table 3: LEM and SE for different traits of single SNP (g. 5465 G→A) of UCP3 gene in Hibird Simmental Cattle

	Birth months				Weaning months				6 months				12 months				18 months			
Genotype	BW (kg)	BH (cm)	BL (cm)	ChG (cm)	BW (kg)	BH (cm)	BL (cm)	ChG (cm)	BW (kg)	BH (cm)	BL (cm)	ChG (cm)	BW (kg)	BH (cm)	BL (cm)	ChG (cm)	BW (kg)	BH (cm)	BL (cm)	ChG (cm)
GG	37.81 ±3.41	72.13 ±1.34	77.53± 1.46	73.91± 3.72	107.6± 11.21	88.76± 4.97	90.96± 2.24	108.96 ±2.56	160.54 ±13.64^{a}	98.68± 3.17^{a}	107.64 ±3.32^{a}	126.18 ±5.49	241.73 ±20.67^{a}	114.67 ±5.32^{a}	132.94 ±4.97^{a}	135.69 ±5.63	442.67± 25.21^{A}	132.14± 10.28^{a}	144.62 ±8.46^{a}	166.21± 10.41^{a}
GA	38.78 ±3.25	74.52 ±1.21	79.75± 1.34	75.68± 3.23	108.8± 10.35	93.17± 3.04	93.37± 2.04	110.17 ±2.36	173.35 ±13.14^{b}	103.21 ±2.91^{b}	112.79 ±3.01^{b}	128.92 ±4.37	256.67 ±20.43^{b}	119.24 ±4.86^{b}	137.31 ±4.65^{b}	137.34 ±5.15	458.84± 23.37^{B}	138.42± 8.54^{b}	151.62 ±7.93^{b}	173.28± 8.21^{b}

^{a,b,c} Values of the different genotype in each column with different lower case superscripts are at $P<0.05$, ^{A,B,C} Values of the different genotype in each column with different upper case superscripts are at $P<0.01$
BW, body weight; BH, body height; BL, body length; ChG, chest circumference

Table 4: LEM and SE for different traits of single SNP (g. 8162 C>T) of UCP3 gene in Hibird Simmental Cattle.

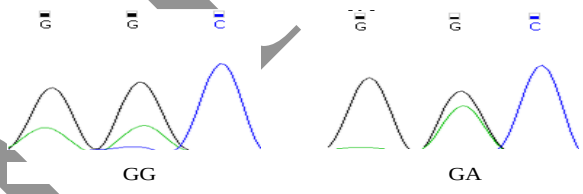
	Birth months				Weaning months				6 months				12 months				18 months			
Genotype	BW (kg)	BH (cm)	BL (cm)	ChG (cm)	BW (kg)	BH (cm)	BL (cm)	ChG (cm)	BW (kg)	BH (cm)	BL (cm)	ChG (cm)	BW (kg)	BH (cm)	BL (cm)	ChG (cm)	BW (kg)	BH (cm)	BL (cm)	ChG (cm)
CC	36.92 ±2.15	71.13 ±3.64	69.47± 3.64	74.89± 3.72	108.23 ±10.64	89.96± 2.12	89.77± 6.24	108.36 ±4.26	170.67 ±15.45	97.68± 8.37^{a}	107.36 ±10.01	124.54 ±6.11^{a}	253.68 ±20.37^{a}	113.27 ±10.16^{a}	133.86 ±8.53	135.45 ±7.96^{a}	454.17± 50.71^{a}	133.14± 16.28^{a}	146.86 ±7.64	170.43± 10.55^{a}
TC	35.47 ±2.32	73.16 ±3.25	74.37± 3.42	72.11± 4.01	107.98 ±10.87	91.02± 1.46	91.56± 2.37	105.79 ±6.94	169.87 ±15.44	106.21 ±2.46^{b}	110.18 ±6.59	120.91 ±8.97^{b}	251.72 ±20.97^{b}	121.85 ±3.28^{b}	135.31 ±6.86	129.62 ±8.48^{b}	450.64± 53.64^{b}	140.42± 5.23^{b}	150.14 ±7.52	163.71± 13.72^{b}

^{a,b,c} Values of the different genotype in each column with different lower case superscripts are at P<0.05, ^{A,B,C} Values of the different genotype in each column with different upper case superscripts are at $P<0.01$
BW, body weight; BH, body height; BL, body length; ChG, chest circumference

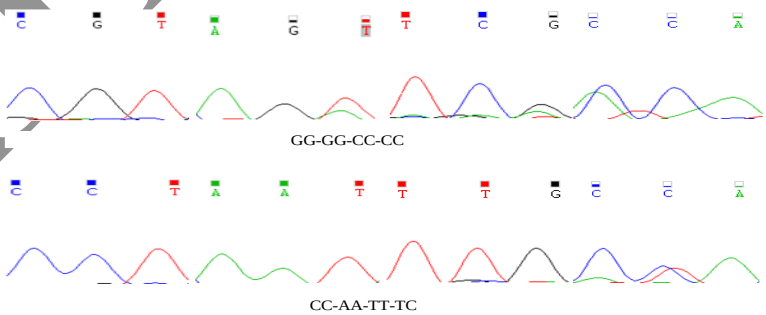
Table 5: LEM and SE for different traits of combined genotypes of UCP3 gene in Hibird Simmental Cattle

		Birth months			Weaning months			6 months			12 months			18 months		
Combinations sites	Combined genotype	BW (kg)	BH (cm)	BL (cm)	BW (kg)	BH (cm)	BL (cm)	BW (kg)	BH (cm)	BL (cm)	BW (kg)	BH (cm)	BL (cm)	BW (kg)	BH (cm)	BL (cm)
5465:g.G→A —8162:g.C>T	GG-CC (n=163)	34.78± 2.34	72.13± 2.56	75.56± 3.55	107.23± 11.35	90.12± 1.23	90.46± 2.56	176.44± 14.35^{a}	104.865 ±9.23	108.36± 4.65	253.78± 19.64^{a}	117.42± 5.16	134.63± 5.33	453.22± 52.64^{a}	139.68± 10.33	148.41± 7.41
	GA-CC (n=143)	35.65± 2.13	72.22± 2.34	77.68± 4.32	109.98± 11.54	91.02± 1.43	91.03± 2.45	185.33± 14.56^{b}	105.32± 8.56	111.35± 5.98	264.53± 19.77^{b}	119.52± 6.41	136.84± 5.46	465.32± 51.67^{b}	140.92± 9.85	150.63± 8.31

^{a,b,c} Values of the different genotype in each column with different lower case superscripts are at P<0.05, ^{A,B,C} Values of the different genotype in each column with different upper case superscripts are at $P<0.01$
BW, body weight; BH, body height; BL, body length



Note: The chromatograms sequencing of the SNP 5465nt (G→A)



Note: The chromatograms sequencing of the SNP 7921nt (G >C), 7953nt (A→G), 8018nt (C>T), 8162nt(T→C)

Fig. 2: The chromatograms sequencing of the fragment at five SNPs

Discussion

UCP3 is a candidate gene for obesity, which is mediated oxidation and ADP phosphorylation process and releases energy by thermal energy form (Zhang *et al.*, 2010). Prior study had displayed that three SNPs (g.820 T>C, g.775 G→A, g.951 T>G) in the exon3 of Qin-chuan bovine, four genotype, AB had superior growth traits (Han and Zhan, 2009). The report showed one SNP (UCP3-BglI, A→G) and three genotype were found in UCP3 gene, AA genotype had greater ChG, BL, BH at 15 months, greater BH at 12 months and greater BL at 8 months (P<0.05) (Chen *et al.*, 2012). Study on Nanyang Cattle UCP3 gene, which found homozygote had better performance than that of heterozygous (Zhang *et al.*, 2010, 2011). In present study, we discovered one novel SNP in the exon3 of UCP3 gene and two genotypes. Individuals with AA genotype had greater growth performance than BB, G5465A mutation affected body weight, body length, body height, especially in later growth period. Besides, we did not find other mutation and genotypes in this locus. These loci have moderate genetic diversity (0.250<PIC<0.500) which indicate that the genetic variation (Hs) are higher. However, our research group was smaller, another reason, heterozygote is disappearing in breed improvement processing. Different breed has some results on UCP3 gene studies, these loci could provide the most powerful genetic markers to distinguish Hybrid Simmental from all other kinds of cattle.

In Previous study, two mutation were found in the exon6 of Simmental, A > G and T > C (Li *et al.*, 2006). In our results, we found two genotypes in the exon6. We couldn't confirm whether or not they have other genotypes. In further studies, extra research with much more cattle groups should be validated. Individuals with AB genotype affected early-gained weight, but 6 months later, individual with AA genotype had greater gain weight, four novel SNPs were identified, g. 7921 C→G, g. 7953 A→G, g. 8018 T→C, g. 8162 T→C, three missense mutations of SNPs altered the encoded amino acid, proline to arginine, cysteine to arginine, tyrosine to histidine, respectively. Four mutations did not report on other breeds by now, therefore we could conclude P2 locus in the exon6 might be used as molecular marker in the future for the growth traits in Hybrid Simmental.

According to present study, we conclude that different genotypes of exon3 and exon6 have significant associated with growth traits, individual with AA genotype has better performance. We recommend using the mutation G5465A, T8162C combined genotypes as a molecular marker for growth traits MAS (Marker Assisted Selection).

Conclusion

In this research, we analyzed the association between five SNPs associated and growth traits in Hybrid Simmental. All the data demonstrate that the polymorphisms of UCP3 could be used as a genetic marker for the cattle breeding of Ningxia. However, the effects of validation of the various allelic effects and functional mechanisms are still exist, also, we need consider that UCP3 variants that already identified in a dependent sample prior or others can be used for marker-assisted selection for the beef cattle.

Acknowledgments

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