African Journal of Biotechnology Vol. 10(54), pp. 11309-11312, 19 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.512
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Polymorphisms in the myostatin gene and their association with growth and carcass traits in duck

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Accepted 25 July, 2011

The objective of this study was to assess the association of polymorphisms in myostatin (MSTN) genes with growth and carcass traits in lines of Peking ducks. The polymorphism of the ducks MSTN gene promoter region was researched by polymerase chain reaction-single-stranded conformation polymorphism (PCR-SSCP) and DNA sequencing methods. Three mutations: G-753A, G-658T and G-235C were detected in the duck MSTN gene. Altogether, 300 Peking ducks of three lines (Z_2 line, Z_4 line and $Z_2 \times Z_4$ cross line) were genotyped and allele frequencies were determined. The effects of MSTN polymorphisms on growth and carcass traits were analyzed. The G-753A was significantly associated with breast meat percentages (BMP) (P = 0.0225). The G-235C indicated significant association with abdominal fat percentages (AFP) (P = 0.0297). No significant association, however, was detected between any of the marker genotype and other traits measured in this study. Results from this study suggest that MSTN gene-specific single-nucleotide polymorphism (SNP) may be a useful marker for growth and carcass traits in future marker assisted selection programs in ducks.

Key words: Duck, myostatin gene, SNPs, PCR-SSCP, growth and carcass traits, association analysis.

INTRODUCTION

Myostatin (MSTN), also known as growth differentiation factor 8 (GDF-8), is a member of the transforming growth factor-β superfamily, which is an essential factor for the growth and development of muscle mass (McPherron and Lee 1997; Mendias et al., 2008). The MSTN gene mainly expresses itself in skeletal muscle and cardiac muscle (Hennebry et al., 2009) and the protein functions as a negative regulator in the process of muscle development in animal breeding (Bellinge et al., 2005), and the polymorphisms of this gene are directly related to the double muscling phenotype (Wiener et al., 2002; Esmailizadeh et al., 2008; Grisolia et al., 2009; Phocas, 2009) which makes it a consolidate candidate gene for the enhancement of productivity in terrestrial livestock and fowl. This shows the meaningful study of the association between the polymorphisms of MSTN and the growth and carcass traits of animals.

The MSTN gene consists of three exons and two intronic regions in many species such as pig (AY208121), buffalo (AH013313), zebra fish (AY323521), gilthead sea bream (AF258447) and chicken (AF346599). All elements of the MSTN gene seem to necessarily have negative effect on muscularity of animals. Loss of function mutations in the MSTN gene coding regions are associated with increased skeletal muscle mass (double muscling) in mice (Mendias et al., 2008), dogs (Mosher et al., 2007), sheep (Clop et al., 2006; Kijas et al., 2007), cattle (Marchitelli et al., 2003) and humans (Schuelke et al., 2004). Besides, the SNPs in promoter, intron and the3'untranslated region of the gene have also been identified to have impact on carcass traits (Kijas et al., 2007; Zhou et al., 2008). Despite the large effects of MSTN mutations on different livestock and fowl breeds, no mutations with major effects have been described in ducks, especially in the promoter region of MSTN gene. By searching "tag" SNPs in the promoter of MSTN in different Peking duck lines (Z_2 line, Z_4 line, $Z_2 \times Z_4$ cross line) in this work, these SNPs could be used to reveal the association between the polymorphisms and the production traits of individuals, which is an increasingly common approach to genetic association studies. The results of this study could add new important evidences that MSTN could be an important candidate gene to be used for the

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Table 1. Primers of the duck MSTN gene designed for PCR-SSCP analysis.

Locus	Primer sequence (5' to 3')[a]	Location[b]	Length (bp)	Annealing temperature (℃)
Frag1	F: ATCTCGTGCAGAAAGCATATTGA R: TGTAAAATGAGTTTGCCTGGTCA	-889~-637	253	55
Frag2	F: GAGATATTTTGAACTGAATTR: R: GTGCATATTTGTCTGTTTGTCC	-769~-561	204	53
Frag3	F: CTTTAGAGAGAGCTCTGCCTTGA R: CCAACAATGAATCTAGCTGTCAG	-331~-88	244	58.2

[a] F is the forward primer and R is the reverse primer; [b] the complete promoter of Pecking duck myostatin gene sequence (GenBank accession no: AY329600) was 1159 bp and the location represents the relative position from the start codon of the MSTN gene.

selection of growth and carcass traits in the duck industry.

MATERIALS AND METHODS

Animals, growth and carcass traits

A total of 300 individuals from three lines of Peking ducks: Z_2 (50♀ and 50♂), Z_4 (50♀ and 50♂) and $Z_2 \times Z_4$ Cross (50♀ and50♂) were derived from the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. All individuals were raised in the same farm and given self-help feeding. The average age was six weeks. Genomic DNA was extracted from whole blood by the standard phenol/chloroform/isoamyl alcohol extraction protocol (Mullenbach et al., 1989), then dissolved in TE buffer (10 mmol/L Tris-HCl and 1 mmol/L EDTA, pH 8.0), and kept at -20℃. Growth and carcass traits measured in the population were six-week weight (WW), eviscerated weight (EW), breast meat percentages (BMP), leg meat percentages (LMP), abdominal fat percentages (AFP) and skin fat percentage (SFP).

Primer design and PCR amplification

Three pairs of primers were designed according to the duck (*Anas platyrhynchos*) MSTN gene (GeneBank accession no: AY329600) to cover the complete region of the MSTN gene promoter. Primers' information is shown in Table 1. PCR amplification was performed in a reaction volume of 20 µl including 50 ng of genomic DNA, 25 pmol of each primer, 0.25 mM dNTPs, 1.5 mM $MgCL_2$ and 1.0 U Taq DNA polymerase. The condition for PCR was as follows: 5 min at 95℃, 35 cycles of 35 s at 94℃, 30 s at different temperature (corresponding to different primer pairs, Table 1), 45 s at 72℃; and a final 8 min extension at 72℃. The PCR products were separated on 1.5% agarose gel (Promega) including 0.5 µg/ml of ethidium bromide, and photographed under UV light.

Genotype determination of PCR-SSCP and DNA sequencing analysis

SSCP method was used to scan mutations within the amplified regions. Aliquots of 5 µl PCR products were mixed with 5 µl denaturing solution (95% formamide deionized, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98℃ and chilled in ice immediately for 5 min. Denatured DNA was subjected to 10% PAGE (polyacrylamide gel electrophoresis) in 1×TAE buffer and constant voltage (140 V) for 10 to 12 h at a constant temperature of 4℃, and then gels were stained with 0.1% silver nitrate. After the polymorphism was detected, the PCR products representing different electrophoresis patterns in different breeds were subcloned to T-vector (Promega) and sequenced in both directions in ABI PRISM 377 DNA sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) by Shanghai Invitrogen Biotechnology Ltd. Co., (Shanghai, P. R. China).

Statistical analysis

Differences in genotypic and allelic frequencies of duck MSTN gene among three lines were analyzed by Popgene32 (Yeh et al., 1997). Data of the breeds were analysed by ANOVA (SAS software GLM procedure) using the following model: $Y_{ijklm}=\mu + Line_i + Age_j + Sex_k + Marker_l + e_{ijklm,}$ where, Y_{ijklm} is the observation of the traits, μ is the least square mean, $Line_i$ is the effect of line, Age_j is the effect of age, Sex_k is the effect of sex, $Marker_l$ is the effect of marker genotype and e_{ijklm} is the residual effect.

RESULTS AND DISCUSSION

Identification of SNPs polymorphism and genotyping

Through PCR-SSCP and DNA sequencing method, three SNPs at -753, -658 and -235 (relative to ATG start codon) of duck MSTN gene, respectively, could be detected. These new allelic variants corresponded to the GA,GT and GC mutations at positions -753, -658 and -235 of duck MSTN gene promoter, respectively. The PCR-SSCP analysis for the three SNPs showed that in the three lines populations, both alleles of G was not predominant. The value of the GG genotype frequency was minimum in all the populations. The genotype frequencies of the MSTN gene SNPs in different populations are shown in Table 2.

Gene-specific SNP marker association analysis with economic traits

Myostatin gene acts as a negative regulation of skeletal muscle growth and keeps the skeletal musculature within appropriate proportions. Comparison of the homology of myostatin demonstrates that myostatin has been highly

Table 2. Genotype frequencies of MSTN gene SNPs in three lines of Peking duck.

SNP	Genotype	Line		
		Z_2 (100)	Z_4 (100)	$Z_2 \times Z_4$ (100)
G-753A	AA	0.43	0.59	0.38
	AG	0.57	0.41	0.57
	GG	0	0	0.05
G-658T	GG	0.22	0.24	0.16
	GT	0.45	0.48	0.41
	TT	0.33	0.28	0.43
G-235C	CC	0.48	0.45	0.66
	GC	0.34	0.50	0.13
	GG	0.18	0.05	0.21

Table 3. Association of the MSTN genotypes and growth and carcass traits in different populations.

SNP	Genotype	N	Traits(LSMEAN ± SE)[a]					
			WW (g)	EW (g)	BMP (%)	LMP (%)	AFP (%)	SFP (%)
G-753A	AA	140	2652.64±14.22	1618.2±12.59	12.49[a]±0.05	17.82±0.15	2.26±0.03	30.82±0.48
	AG	155	2611.12±16.22	1590.1±13.47	10.97[b]±0.03	17.83±0.14	2.40±0.04	31.76±0.46
	GG	5	2568.76±21.79	1540.1±17.41	10.46[b]±0.13	17.86±0.23	2.42±0.10	31.86±0.96
	P		0.5613	0.5821	0.0225	0.7662	0.1232	0.2687
G-658T	GG	62	2807.27±21.66	1620.3±13.44	10.24±0.10	2.46±0.04	2.46±0.05	32.79±0.56
	GT	134	2736.15±28.64	1587.3±15.36	10.05±0.11	2.55±0.02	2.62±0.04	32.46±0.45
	TT	104	2672.16±25.61	1538.3±11.38	9.65±0.08	2.62±0.07	2.55±0.04	33.64±0.51
	P		0.1178	0.0485	0.5704	0.4850	0.7754	0.6410
G-235C	CC	159	3171.34±27.17	1880.7±12.57	11.51±0.16	16.77±0.17	2.43[a]±0.03	29.57±0.46
	CG	97	3091.52±25.52	1820.7±12.54	11.41±0.12	16.67±0.18	2.48[a]±0.06	30.16±0.66
	GG	44	3010.08±19.67	1781.9±13.42	11.28±0.11	16.58±0.19	2.56[b]±0.02	30.25±0.73
	P		0.4978	0.9171	0.1400	0.9813	0.0297	0.1468

[a]Least squares means value ± standard errors; WW, 6-week weight; EW, eviscerated weight; BMP, breast meat percentages; LMP, leg meat percentages; AFP, abdominal fat percentages; SFP, skin fat percentage. Different letters ([a] and [b]) denote extremely significant differences between groups ($P < 0.05$).

conserved during animal evolution. The predicted amino acid sequence has an overall similarity with a comparable region of Turkey (99%), domestic goose (98%), chicken (99%), human (92%) and pig (92%). Investigations of myostatin develop- mental expression in skeletal muscles (Shibata et al., 2003) and its function in myogenesis and adipogenesis showed that myostatin expression is related to animal growth (Lin et al., 2002; Joulia et al., 2003; Rebbapragada et al., 2003; Wagner et al., 2005). Mutations in MSTN pro- moter could lead to changes of the gene expression and thereby influence growth and development. Some research results showed that the lack of MSTN gene strongly affects muscle phenotype in humans and other animal species (Joulia-Ekaza and Cabello, 2007). Piedmontese crossbreds with an inactive MSTN allele have higher birth weights and yearling weights (Casas et al., 1999). In mice, the MSTN gene knockout causes a significant increase in muscle mass through muscle cell hypertrophy and hyperplasia (Grobet et al., 2003; Whittemore et al., 2003; Tang et al., 2007; Welle et al., 2007). There is no similar research about the association analysis of the MSTN gene with growth and carcass traits in duck. In this study, the MSTN gene is considered to be one of the potential candidate genes influencing growth and carcass quality traits in duck; the gene-specific SNP markers association analysis were evaluated and shown in Table 3. The -G753A SNP marker was significantly associated with BMP.

Individuals with the AA genotype indicated significant association with higher BMP than AG and GG genotype (P = 0.0225). The pattern of myostatin expression was closely parallel to the trend of breast muscle growth, suggesting that myostatin might play an important role in breast muscle development. It was postulated that myostatin may be a major determinant of muscle mass in breast muscle, as also shown in other species. Significant association between the G-235C locus and AFP was found. Ducks of genotype CC had significant lower AFP than those of genotype CG and CC (P = 0.0297). No significant association, however, was detected between the marker genotypes and growth and carcass traits measured in this study (Table 3). Results from this study show that the mutations in the promoter region of the duck MSTN gene were important reasons for the variance of growth and carcass traits, opening up possibilities for duck breeding and improvement in gene-assisted selection. Therefore, the MSTN gene could act as a candidate gene for the duck breeding and industry.

REFERENCES

Bellinge RHS, Liberles DA, Iaschi SPA, O'Brien PA, Tay GK (2005). Myostatin and its implications on animal breeding: a review. Anim. Genet., 36: 1–6

Casas E, Bennett GL, Smith TPL, Cundiff LV (2004). Association of myostatin on early calf mortality, growth, and carcass composition traits in crossbred cattle. J. Anim. Sci., 82: 2913-2918

Clop A, Marcq F, Takeda H (2006). A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nat. Genet., 38: 813–818

Esmailizadeh AK, Bottema CD, Sellick GS, Verbyla AP, Morris CA (2008). Cullen NG, Pitchford WS, Effects of the myostatin F94L substitution on beef traits. J. Anim. Sci., 86: 1038–1046

Grisolia, AB, Dangelo GT, Porto Neto LR, Siqueira F, Garcia JF (2009). Myostatin (GDF8) single nucleotide polymorphisms in Nellore cattle. Genet. Mol. Res., 8: 822–830

Grobet L, Martin LJ, Poncelet D (1997). A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nat. Genet., 17: 71–74

Hennebry A, Berry C, Siriett V, O'Callaghan P, Chau L, Watson T, Sharma M, Kambadur, R (2009) Myostatin regulates fiber-type composition of skeletal muscle by regulating MEF2 and MyoD gene expression. Am. J. Physiol. Cell. Physiol., 296: 525–534

Joulia D, Bernardi H, Garandel V, Rabenoelina F, Vernus B , Cabello G (2003). Mechanisms involved in the inhibition of myoblast proliferation and differentiation by myostatin. Exp. Cell. Res., 286: 263-275

Kijas JM, McCulloch R, Hocking Edwards JE, Oddy VH, Lee SH, van der Werf J (2007). Evidence for multiple alleles effecting muscling and fatness at the ovine GDF8 locus. BMC Genet., 8: 80

Lin J, Arnold B, Della-Fera MA, Azain MJ, Hartzell DL, Baile CA (2002). Myostatin knockout in mice increases myogenesis and decreases adipogenesis Biochem Biophys. Res. Commun., 290:701-706

Marchitelli C, Savarese MC, Crisa A, Nardone A, Marsan PA , Valentini A (2003) .Double muscling in Marchigiana beef breed is caused by a stop codon in the third exon of myostatin gene. Mammalian Genome, 14: 392–395

McPherron AC, Lee SJ (1997). Double muscling in cattle due to mutations in the myostatin gene. PNAS, 94: 12457–12461

McPherron AC, Lawler, AM, Lee, SJ (1997). Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature, 387: 83–90

Mendias CL, Bakhurin KI, Faulkner JA (2008 Tendons of myostatin-deficient mice are small, brittle, and hypocellular. PNAS, 105: 388–393

Mosher DS, Quignon P, Bustamante CD, Sutter NB, Mellersh CS, Parker HG , Ostrander EA (2007). A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygous dogs. PLoS Genet., 3: e79

Phocas F (2009).Genetic analysis of breeding traits in a Charolais cattle population segregating an inactive myostatin allele. J. Anim. Sci. 87: 1865–1871

Rebbapragada, A, Benchabane H, Wrana JL, Celeste AJ , Attisano L (2003). Myostatin signals through a transforming growth factor [beta]-like signaling pathway to block adipogenesis. Mol. Cell .Biol., 23: 7230-7242

Schuelke M, Wagner KR, Stolz LE, Hu¨ bner C, Riebel T, Ko¨men W, Braun T, Tobin JF , Lee SJ (2004). Myostatin mutation associated with gross muscle hypertrophy in a child. N. Engl. J. Med., 350: 26822688

Shibata M, Ohshima K, Kojima T, Muramoto T, Matsumoto K, Komatsu M, Aikawa K, Fujimura S ,Kadowaki M (2003). Nucleotide sequence of myostatin gene and its developmental expression in skeletal muscles of Japanese Black beef. J. Anim. Sci., 74: 383-390

Wagner, KR, Liu X, Chang X and RE Allen (2005). Muscle regeneration in the prolonged absence of myostatin. PNAS, 102: 2519-2524

Wiener P, Smith JA, Lewis AM, Woolliams JA, Williams JL. (2002). Muscle-related traits in cattle: the role of the myostatin gene in the South Devon breed. Genet. Sel. Evol., 34: 221–232

Zhou H, Hickford JGH, Fang Q (2008). Variation in the coding region of the myostatin (GDF8) gene in sheep. Mol. Cell. Probes, 22: 67–68

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997). POPGENE,the user-friendly shareware for population genetic analysis. Edmonton, Alberta, Canada, Molecular Biology and Biotechnology Centre, University of Alberta, Canada (http://www.ualberta.ca/*fyeh/info.htm)