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Identification of Genetic Variants Within Androgen Receptor Gene of Sika Deer and its Association with Antler Production

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Abstract: Antler production is one of the most important economical traits of Sika deer. However, the genetic mechanism of antler growth and genetic markers associated with antler yield remain unclear. In the present study Androgen Receptor (*AR*) gene has been considered as a candidate gene to identify the polymorphisms. Besides, its effect on antler production was investigated in Chinese Sika deer. Genomic sequences of exons1-7 of Sika deer have been successfully obtained and showed high homogeneity with bovine. One SNP ss325995755 was identified in exons3. Genotyping of SNP and association analyses with antler yield were analyzed in two Chinese Sika deer populations (n = 215). The SNP ss325995755 was not significantly ($p>0.05$) associated with average antler yield.

Key words: Androgen receptor gene, antler growth, association, Sika deer, significantly

INTRODUCTION

Sika deer is classified into Mammalia, Artiodactyla, Cervidae, Cervu. The ancestries of *Cervidae* species were earliest originated at Oligocene of the Tertiary Period and Sika deer was mostly possibly derived from North China at the late of Pliocene Epoch which was >3 million years ago (Corbet and Hill, 1991). China is one of the three largest productive countries in the world and has a long deer breeding history which could date back from Shang Dynasty in 11BC (Dong *et al*., 2000). Domestic deer industry has developed rapidly and till now there are more than thousands of deer farms in China. The total number of Sika deer is >300 thousands.

Antler is the most important products of deer farming and has great economic values because it is one of the precious Chinese medicines and has various kinds of medical and health care roles. Antler is one of the fastest growing parts of deer body. It has round end, fuzzy coat and full of connective tissue and cartilaginous tissue inside. The previous researches have demonstrated that antler size was associated an individual's nutritional state (Kruuk *et al*., 2002). Antler production was significantly related to the deer's live weight (Zeng *et al*., 2002). Besides, antler formation and growth rate are also, related to sex hormones including progesterone, thyroid hormone, adrenocortical hormone, chorionic gona-dotropin (Sempere and Lacroix, 1982; Li, 2003; Gao, 1999; Bartos *et al*., 2009; Bubenik *et al*., 1987, 1997).

Androgen is important for male reproductive organ development and function maintenance of humans and animals. Androgen Receptor (AR) is one of type I steroid receptors and is a ligand-dependent transregulator protein. Androgen concentrations were low and antler growth had commenced and was proceeding rapidly in the velvet from March through July (Mirarchi *et al*., 1977). The antler cycle of Southern pudu is very sensitive to manipulation of androgen levels (Bubenik *et al*., 2002). Recently, a few of molecular studies and association analyzes between antler growing trait and candidate genes such as Growth Hormone (GH) and Major Histocompatibility Complex (MHC) have been carried out (Swarbrick *et al*., 1995; Du and Bai, 2004). Results showed an effect of *GH* gene on antler production. Deer with the SNP genotypes G→A had a significant difference in antler production of the fifth saw ($p<0.2$) (Du and Bai, 2007). Nine alleles of the second exon of *MHC-DRB* gene that are significantly positively correlated to the antler produetivity were diseovered (Li, 2003). These results showed that screening more candidate genes related to antler growing is very important. In this study, *AR* gene

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was considered as one of candidate genes and the association between AR genotypes and antler growth traits were explored so as to find the potential molecular markers related to antler growing which can be used for Marker Assisted Selection (MAS) programs in Sika deer industry.

MATERIALS AND METHODS

Animals: All procedures involving animals were approved by the Animal Care and Use Committee of Huazhong Agricultural University. A total of 215 Sika deers were examined in this study which were obtained from 2 deer farms including Wusan Sika deer farm (n = 136, Jingmen city, Hubei Province), Dongfeng county Sika deer farm (n = 79, Jilin province). Approximate 10 mL blood per Sika deer was collected, aseptically from the jugular vein and kept in a tube containing anticoagulant EDTA (Ethylenediaminetetraacetic acid). All samples were delivered back to the laboratory in an ice box. The genomic DNA was extracted from white blood cells using standard phenol-chloroform extraction protocol (Joseph and David, 2002). The DNA samples were dissolved in TE buffer which was made from 10 mM Tris-Cl (pH 7.5) and 1 mM EDTA (pH 8.0) and were stored at -20°C for use. The animals from Shuangyang Sika deer and Dongfeng Sika deer had the detailed records of antler harvest time and fresh weight.

Mutation detecting and genotyping: A total of 8 pairs of primers were designed using Primer 5.0 (PREMIER Biosoft Inc, Palo Alto, CA, USA) on the basis of DNA sequence of the bovine AR (Accession: NC007331, Z75313, Z75314, Z75315) (Table 1). The primers were synthesized by Sangon Biotech Company (Shanghai, China). Both PCR reactions were performed in a 10 μL mixture containing 1× PCR buffer, 0.2 uM each primer, 150 uM each dNTP, 1.5 mM $MgCl_2$, 2U Taq DNA polymerase (Takara Company, Dalian, China) and 12.5 ng genomic DNA. PCR reactions were comprised of the initial denaturation at 95°C for 5 min, 30 cycles with 94°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec followed by a final extension at 72°C for 5 min. These sequences were then blast with bovine *AR* gene sequence to obtain the homology between species. PCR-SSCP Method was used to detect the polymorphisms. Two-direction sequencing of the amplicons was implemented by BGI Ark Biotechnology Company (Shenzhen, China) to detect the mutations.

Table 1: The primer sequence for Sika deer *AR* gene

Primer sequence (5'→3')	Annealing temp. (°C)	Product size (bp)	Region amplified
TTTCAGAGTGTATGCGAAGTGA	57.0	218	exon1*
ATGTTGTGAAGGCTGCTGTT			
CCCTGGAAGAGGAACAGCA	52.2	225	exon1**
TGTCTTTAAGATCGGTGGAGC			
ATTATTCCTCTTGGGTCT	56.5	284	exon2
ACTGCCAGTGACTTTGTC			
TTAACAGGGCTGTCTACT	54.1	163	exon3
GTATCATACCTCCCAGAG			
TAAATCCAACTTTCCCTTCT	58.5	159	exon4
GCTGACACTCATAGCCTTC			
CAGTCTGACCATTGCCTGTG	51.5	236	exon5
CCGTTATCGCCATGAACC			
TACCCCTCTCTTTTCTCTGTGTGTT	57.6	183	exon6
CCCTTCCAGGCACTTACT			
CCCCATTCTGTCTTCATC	57.1	205	exon7
ACCCTCCATTGTTTGCTT			

*Indicates one primer of exon1; **Indicates another primer of exon1

Data statistics: The association analyses were implemented using GLM Model procedure (SAS 9.0; SAS Institute, Cary, NC, USA). The model was showed as follows:

$$P_{ijk} = \mu + G_i + Q_{ik} + e_{ijk}$$

Where:

P_{ijk} = The different saw antler harvest capability of animal
μ = The population mean
G_i = The fixed effect of genotype
Q_{ik} = The fixed effect of different farm
e_{ijks} = The random residual error

RESULTS AND DISCUSSION

Genotypes and diversities: About 8 pairs of primers of the *AR* gene were amplified from Sika deer genomic DNA as expected (Fig. 1). PCR-SSCP method was used to detect the polymorphism of *AR* gene exon1-7 (Fig. 2). These results showed that there was no genetic variation in exon1-7 while 1 mutation was identified in exon3 (T75C). The mutation was submitted to the GeneBank and acquired the dbSNP Accession No.: ss325995755 and then the exon3 sequence was submitted to Genebank (Accession: JF719040). The transition of T75C caused an amino acid change from Val to Ala. However, The SNP destroyed the restriction site recognized by endonuclease Rsal. These following DNA restriction fragments were generated by the AR-Rsa/polymorphisms: 88, 49 and 26 bp for CC genotype, 114 and 49 bp for TT genotype, 114, 88, 49 and 26 bp for CT genotype (Fig. 3). At T75C locus, 194 deers were homozygous (CC), 5 deers were heterozygous (CT) and 16 deers were homozygous (TT) (Table 2). In the tested Sika deer population, 3 kinds of genotypes were found in Dongfeng Sika deer populations. Nevertheless, only CC and TT homozygote genotype were observed in Wusan Sika deer populations. Allele C was predominant in all of populations.

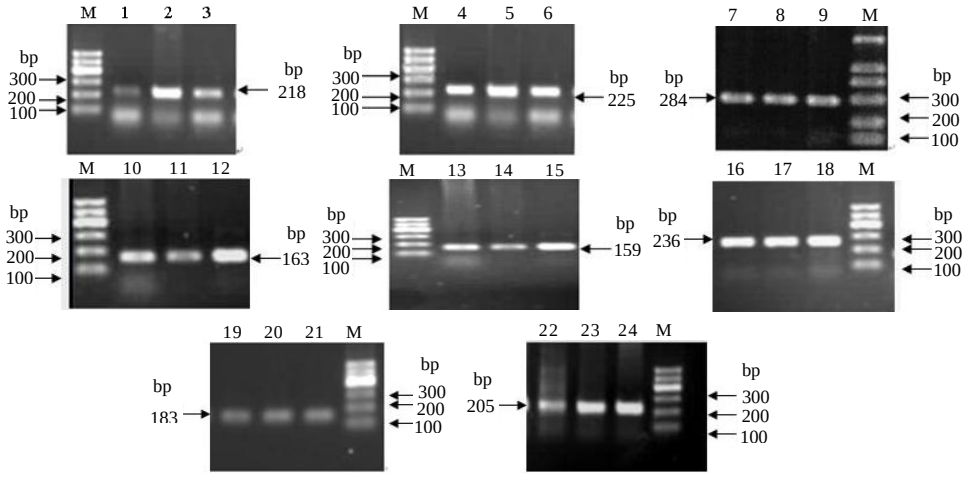

Fig. 1: Electropherotic profile for the AR fragments amplified by PCR in a 1.2% (w/v (5V/cm) agarose gel. A stand with 218 bp (Lanes 1-3), 225 bp (Lanes 4-6), 284 bp (Lanes 7-9), 163 bp (Lanes 10-12), 159 bp (Lanes 13-15), 236 bp (Lanes 16-18), 183 bp (Lanes 19-20) and 205 bp (Lanes 22-24) appeared to represent amplicons using primer exonl*, exonl**, exon2-7, respectively. M represented a Marker I (600, 500, 400, 300, 200 and 100 bp)

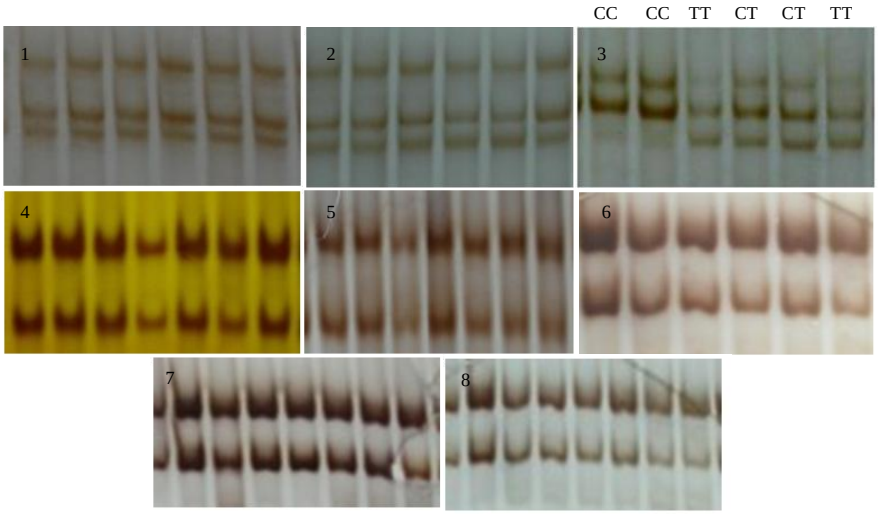

Fig. 2: PCR-SSCP for the genotype of *AR* gene exonl-7, 1-8 represent primer exonl*, exonl**, exon2-7, respectively

Table 2: The genotypic and allelic frequencies of the androgen receptor gene in Sika deer

Genotype	Genotype frequency	Allele frequency
CC (194)	0.90	C 0.92
CT (5)	0.03	T 0.08
TT (16)	0.07	-

Association of genotypes with antler yield traits: Association analyze showed that there was no significant differences between antler yield and the genotypes ($p>0.05$) at saw 1-5 (Table 3). However, there was a tendency that CT genotype individuals had worse performance in yield traits than CC and TT genotype. The biological procedure of antler formation, growth and regeneration of Sika deer is complicated and there is little known about the molecular mechanism regulating this procedure. At present, most of molecular studies are on association between antler growing trait and candidate genes such as Growth Hormone (GH) and Major Histocompatibility Complex (MHC). In this study, exons sequencing, genotyping and association of Sika deer *AR* gene were implemented. It was found that a non-synonymous mutation in exon3. Association analyses showed that there was no significantly association between average antler yield and genotypes ($p>0.05$). However, CT genotype individuals were lower in yield traits than CC and TT individuals from the 1st saw to the

Table 3: The association of genotypes with antler yield of different saw in Sika deer

	Antler yield (g)±SD				
Genoytpe	Saw 1	Saw 2	Saw 3	Saw 4	Saw 5
CC (194)	519.82±61.55	1098.27±96.87	1753.99±86.89	1910.27±65.74	2392.84±91.80
CT (5)	402.84±166.45	724.59±376.66	1440.91±432.74	1334.15±359.14	1909.84±745.25
TT (16)	400.95±157.28	1082.50±316.10	1861.46±304.34	1963.18±229.35	2469.54±277.13

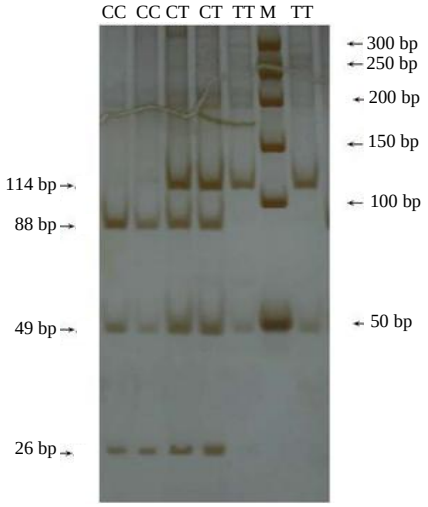

Fig. 3: Representative genotyping of *AR* gene at locus T75C by polyacrylamide gel electrophoresis. Stands with 88, 49 and 26 bp for CC genotype, 114 and 49 bp for TT genotype, 114, 88, 49 and 26 bp for CT genotype appeared at this locus. M represented a market with 50 bp DNA Ladder/RSA I (500, 400, 300, 250, 200, 150, 100 and 50 bp)

5th saw. It was consistent with the genotype frequency that CT was the lowest. It suggested that this locus was possibly associated with the major gene regulating the antler yield. The CT individuals may be eliminated by artificial selection as the poor performance. In the previous studies in Finnish men at the beginning of the CAG repeat in the exon1 of the androgen receptor gene the 173A-->T (Q58L) substitution might be the cause of infertility (Lund *et al*., 2003) in human, exon7 of the androgen receptor gene, Gln824Lys mutation in the ligand-binding domain of to cause slight impairment of receptor function but was compatible with preservation of male fertility (Giwercman *et al*., 2000).

In this study, 2 pairs of primers was designed based on exon1 sequence, no genetic variant was identified. The possible reasons are relatively small sample size of the analyzed population and alleles have been fixed due to long term artificial selection. This study also implied that *AR* gene may involve in the growth and development of antler of Sika deer. In the future research, SNP detection could be implemented in other un-detected coding region and introns. Additionally, enlarged populations with different genetic background are also warranted.

CONCLUSION

In the present study, genomic sequences of seven exons of Sika deer were obtained using comparative homologous amplification. SNP from exon3 of androgen receptor of Sike deer including a non-synonymous mutation GTA (Val)/GCA (Ala) were identified in Chinese Sika deer population. PCR-RsaI-RFLP was developed for genotyping in two Sika deer populations. The result showed that there was no significantly ($p>0.05$) association with the antler yield. However, there was a tendency that CT genotype individuals had worse performance in yield traits than CC and TT genotype. Therefore, the *AR* gene could be considered as a candidate gene for antler production of Sika deer. Besides, additional causative mutation discovery and association implication are warranted.

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