Identification of SNP markers associated with antler growth rate in sika deer (*Cervus nippon*)

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Brief report

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Abstract

Sika deer (*Cervus nippon*) constitutes one of the most valuable animal genetic resources in east Asia. The aim of this study was to identify and validate single nucleotide polymorphisms (SNPs) from antler growth-related genes of sika deer. The whole genome sequencing data of sika deer were used to identify SNP markers. Among them, 31 SNPs from antler growth-related genes exhibited significant polymorphism using genotyping by mass spectrometry. The observed and expected heterozygosities were ranged from 0.147 to 0.997 and 0.201 to 0.500, respectively. Significant deviation from the Hardy-Weinberg equilibrium was observed in 6 loci. These findings provide effective molecular detection markers for the study of variation in antler growth rate of sika deer.

Background

Sika deer (*Cervus nippon*) were once widely distributed in eastern Asian countries, and this species is one of the most commercially valuable species for production of high value velvet antler for medicinal use (Hu et al. 2019). There are large herds of farmed animals kept mainly for production of velvet antlers where the antler growth rate is the key factor that determines the profitability of deer farming. Therefore, the identification of molecular markers associated with antler growth-related genes offers potential for application in the selection of sika deer (especially females who do not grow antlers) for artificial breeding.

Single nucleotide polymorphisms (SNPs) have been widely used for investigating genetic diversity, species identification and for exploring potential applications in breeding for deer (Ba et al. 2017), cattle (Zhang et al. 2019) and goats (Ee et al. 2019). Using the genome sequencing data of the sika deer individuals with fast and slow growing velvet antler status (Genbank accession number PRJNA541418), we have obtained a large number of SNPs.

We utilized the mass spectrometry SNP detection technology combined with multiplex PCR, single base extension and Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) to identify SNP variation in individual deer. This technology offers advantages as up to 40-fold multiplex PCR reactions and genotype detection can be run at one time, and can be used simultaneously on thousands of samples at numerous SNP sites, with an accuracy of up to 98% (Colombo et al. 2019; Jiménez-Sousa et al. 2019).

The genomic and transcriptomic sequencing data of fast growing velvet antlers compared with slow growing velvet antlers were analyzed and antler growth-related genes were identified. The gene sequence containing SNP sites was extracted from the sequencing data of our sika deer genome, and a total of 31 SNPs were selected. In order to validate the polymorphisms at these SNP loci, PCR reactions and single base extension primers were designed using the software MASARRAY assay design suite v3.1 (Table 1). Genomic DNA was extracted from peripheral venous blood of farmed sika deer (from northern China) using whole blood genome DNA isolation kit (BioTeke Corporation, Beijing, China). The PCR amplification, shrimp alkaline phosphatase (SAP) digestion, and extension reaction were carried out sequentially; the reaction product (9 µl in total) was diluted 3 times, desalinated with resin, and the desalted sample was placed on the sample target and allowed to crystallize naturally. Mass spectrometry detection was carried out and data collected. The original results were sorted out and detection rate of each SNP loci was calculated. Population genetic indexes including expected heterozygosity (He), observed heterozygosity (Ho), Hardy-Weinberg equilibrium (HWE) and Minor Allele Frequency (MAF) were calculated using Haploview 4.1 software.

Samples from a total of 384 farmed sika deer were analyzed. The detection rate for the 31 SNP loci was ranged from 83% to 100%. There was a high degree of polymorphism among the 31 loci and the MAF was ranged from 0.113 to 0.499. The He and the Ho were ranged from 0.201 to 0.500 and from 0.147 to 0.997, respectively. Significant deviation from Hardy-Weinberg equilibrium was observed in 6 loci (Table 2).

Our findings provide the basis for the investigation of the potential for the application of molecular markers in the selection of sika deer for breeding.

Declarations

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Tables

Table 1 Primer sequence and product length used in SNPs verification

Gene	1st-PCR primer sequence	2nd-PCR primer sequence	Single base extension primer sequence Product length(bp)	
PIGN	ACGTTGGATGCAGTTCAGTTCATTAGTAG	ACGTTGGATGAACTCTGGGAGTTGGTGATG	ACGCAAAGAGTCGGACAC	119
RPL12	ACGTTGGATGTTCCACATCCCTTCAGTTTG	ACGTTGGATGAGCTGTTACAGGCAGATCTC	CTCTACTGCACTTCACAGAGAGTGAG	104
ATF6	ACGTTGGATGACTTGAACCTCCTCCTCCAG	ACGTTGGATGGACAGATAGCTAATGGGAAG	TAGCTAATGGGAAGCTGCTG	119
ARPC1A	ACGTTGGATGCATGCTTTTTGCTGGCTTCC	ACGTTGGATGGACCAGGATTCAAGTGCAAG	CCTGAGAGGTGGCAGAG	100
FGFR2	ACGTTGGATGGTTTATTGGGAGCAGGTATC	ACGTTGGATGTAATGTGGTCCATGGGAAGG	GGATGAGAGATACTTCTTTTG	107
ECPAS	ACGTTGGATGAACTCTTCATCGGTAACCTC	ACGTTGGATGGCATAAGTCAACCAGTACCC	GCACTATACAAGCATTCCAT	92
CTSC	ACGTTGGATGAAGGACAGACTTGTCATGCG	ACGTTGGATGCTCTGGAGGAGTAAAGTAGG	AGTAGGTTGGCATAACTT	101
NPM1	ACGTTGGATGTGAGCGCTGACAATGACACC	ACGTTGGATGAAGGCCAGGACCTAGCCGA	GAGCCTGGCGGCAGGAACAATG	119
RPS20	ACGTTGGATGTGGATGGAGGTAAGAATGGG	ACGTTGGATGGGGTGGGATGGAAGGGTTA	GGAAGGGTTAGGTGGA	103
ATP1A1	ACGTTGGATGTGGTACAGAGACACTTGAGG	ACGTTGGATGTTTACAGCCCTCAGAGAAGGC	GGGACGCAGCCGCAGCTGT	125
SPPL2A	ACGTTGGATGTGTACTACTAAGCCTCTGCC	ACGTTGGATGGGTCAAATAGCAGCAATTC	GGGTTTCTTGTGGTGATG	80
TRPM7	ACGTTGGATGGCAGTAACAGTCTGTTTGCC	ACGTTGGATGGGCTTAGTTACCTACTTTCC	GACAGTTTGTAGTACACTAGGAATGA	101
NPC1	ACGTTGGATGCAGTCCCAATGACCGGAATT	ACGTTGGATGAATAAAGTCTGGCCGATGC	GGGTGCTGTGGCCATTTGGA	86
PDCD6IP	ACGTTGGATGTCTGAATCCCATACAGAAGG	ACGTTGGATGCTTGGAGCAGTTGAATAGCC	CCCCAGTTGAATAGCCATTTCCCA	120
ARPC2	ACGTTGGATGGCCCCAAGAGAAAAAAGG	ACGTTGGATGTTAAAGCCAATCAACCCACC	AACCCACCAACCCAG	125
YTHDF2	ACGTTGGATGGAATCAGATGACCCTGCTTG	ACGTTGGATGAGTAAGGCTGTGTAATGCTC	TCATAGATTTGGGCAGTCA	94
BAIAP2	ACGTTGGATGACGGCTTTCTGTGCTCATGG	ACGTTGGATGAAGGTGCAGCTCTGCACAG	TGGACCCAGATAGCA	127
IGF1R	ACGTTGGATGAAAGTTGCTTCTGCTGACC	ACGTTGGATGCAATGAGTGTGTCCATGACG	CACACGGCGGGAGTGC	111
ITGB5	ACGTTGGATGTCTGCAGCCAGGCTACATC	ACGTTGGATGTCTGTTCTCCAGCATCAATC	GAACCACAGCCCCAGAGGCA	100
NCAM1	ACGTTGGATGAATGAGAGAGACAGTTTGGC	ACGTTGGATGACCCGTGGAATTGATCTGAC	TACAACATGTTTAGAGGAAATTATA	153
CCNY	ACGTTGGATGTGTTGTTGCAAATGGCAGGG	ACGTTGGATGGGGTAAAGGAAATGTGAGAG	AAATGTGAGAGATAGATACAAG	112
MSRB3	ACGTTGGATGACCCAGTAAAGCAATGGCAG	ACGTTGGATGGTGACATTTCCATTGCCAC	ACGTCCTGGTGACCATAACATAAATC	99
AOPEP	ACGTTGGATGTTTCATGCATCTGACAGGCG	ACGTTGGATGGGAAATGAAACGGCTAAGG	CTAAGGACATATGAAAGCTCA	116
RPL7	ACGTTGGATGCCTGCTAAAGCTGACTCAAG	ACGTTGGATGCAACCATCTATTTGGTTGGC	CCTGTGGTTGGCCAAATATTCTTT	125
B4GALT1	ACGTTGGATGACAACTGAGGCACAGAGAG	ACGTTGGATGCCTGTCTTCAGATCCTGTTG	TCGCTGCTTCTTCCAGTTTCA	100
FMNL2	ACGTTGGATGAGGTCAAACCACCACTAACG	ACGTTGGATGATTGTCTAGGCCTCACAGTC	GTTGTGGTGATGGGGTGCTTG	105
STAM2	ACGTTGGATGTTTGAGTTGGCTAGGGATGG	ACGTTGGATGTATGCTTTTGGTGCTCTCC	TGTAATCCCTGGTCTCCTT	84
GUCY1B1	ACGTTGGATGACAGAACTGGCTCACAGAC	ACGTTGGATGCTCCTAATTTATCCCTCCCC	TTTATCCCTCCCCAACCCACTTTC	101
CHFR	ACGTTGGATGTGTGGTTTACAGACAGCGAG	ACGTTGGATGTTCTTACACAAACGGGTGGC	GGTGGCTACTTCCCTG	96
ENAH	ACGTTGGATGACCTGGAAGTCCTTCCCTC	ACGTTGGATGAACAGTGACAGTGGAGATGG	TGGAGATGGTTTGGCG	124
RAB3GAP2	ACGTTGGATGGAGTCTGTTTCATCAGCATCC	ACGTTGGATGGTAGTCCCTGACCTCGTATA	GTCCCTGACCTCGTATAAATCTAAG	105

Table 2 Summary of the 31 SNP markers in the farmed sika deer

Gene	Site	SNP	Position	Detection rate	MAF	Ho	He	P _{HWE}	Function
IGF1R	A11323595G	A/G	intronic	97.1	0.403	0.147	0.481	0.000	Insulin-like growth factor 1 receptor
ARPC1A	G666285T	G/T	intergenic	99.7	0.482	0.963	0.499	0.000	Actin-related protein 2/3 complex subunit 1A
SPPL2A	C4458043T	C/T	intronic	85.2	0.384	0.731	0.473	0.000	Signal peptide peptidase-like 2A
PDCD6IP	G6429786T	G/T	intronic	99.7	0.193	0.193	0.312	0.000	Programmed cell death 6-interacting protein
PIGN	A10067G	A/G	intronic	97.7	0.499	0.997	0.500	0.000	GPI ethanolamine phosphate transferase 1
NCAM1	A16125736G	A/G	intronic	93.0	0.249	0.230	0.374	0.000	Neural cell adhesion molecule 1
FGFR2	A950852T	A/T	intergenic	96.1	0.369	0.417	0.465	0.058	Fibroblast growth factor receptor 2
ATF6	A349957G	A/G	intronic	96.6	0.283	0.410	0.406	0.983	Cyclic AMP-dependent transcription factor ATF-6 alpha
ATP1A1	A4017558C	A/C	downstream	100	0.299	0.438	0.420	0.492	Sodium/potassium-transporting ATPase subunit alpha-1
ARPC2	A9730945G	A/G	intronic	86.2	0.437	0.462	0.492	0.310	Actin-related protein 2/3 complex subunit 2
RPL12	C176548G	C/G	intergenic	98.2	0.241	0.355	0.366	0.640	60S ribosomal protein L12
ECPAS	A1394979G	A/G	intronic	99.7	0.497	0.509	0.500	0.818	Proteasome adapter and scaffold protein ECM29
CTSC	A1466419C	A/C	intronic	99.5	0.301	0.366	0.421	0.016	Dipeptidyl peptidase 1
NPM1	C3449425T	C/T	intronic	86.2	0.196	0.320	0.316	0.966	Nucleophosmin
RPS20	G3550486T	G/T	intergenic	99.5	0.223	0.340	0.346	0.831	40S ribosomal protein S20
TRPM7	C4511966T	C/T	intergenic	97.9	0.117	0.202	0.207	0.803	Transient receptor potential cation channel subfamily M member 7
NPC1	C4874303T	C/T	intronic	99.7	0.275	0.373	0.399	0.246	NPC intracellular cholesterol transporter 1
YTHDF2	C11089790T	C/T	intergenic	98.4	0.190	0.323	0.308	0.482	YTH domain-containing family protein 2
BAIAP2	A11133199G	A/G	intronic	100	0.203	0.307	0.324	0.385	Brain-specific angiogenesis inhibitor 1-associated protein 2
ITGB5	C11629896T	C/T	intronic	99.5	0.355	0.474	0.458	0.585	Integrin beta-5
CCNY	A18285014G	A/G	intergenic	98.7	0.113	0.201	0.201	1.000	Cyclin-Y
MSRB3	A19289133G	A/G	intronic	99.2	0.425	0.525	0.489	0.189	Methionine-R-sulfoxide reductase B3
AOPEP	C19630193T	C/T	intronic	99.2	0.266	0.375	0.391	0.499	Aminopeptidase O
RPL7	C23029824T	C/T	intergenic	99.7	0.377	0.462	0.470	0.809	60S ribosomal protein L7
B4GALT1	C25769614T	C/T	intergenic	99.0	0.303	0.400	0.422	0.354	Beta-1,4-galactosyltransferase 1
FMNL2	C36393203T	C/T	intronic	99.5	0.382	0.482	0.472	0.799	Formin-like protein 2
STAM2	C36759498T	C/T	intergenic	100	0.486	0.570	0.500	0.008	Signal transducing adapter molecule 2
GUCY1B1	C38343077T	C/T	intronic	82.8	0.121	0.192	0.213	0.138	Guanylate cyclase soluble subunit beta-1
CHFR	C39032898T	C/T	upstream	96.6	0.144	0.251	0.247	0.978	E3 ubiquitin-protein ligase CHFR
ENAH	A50172608G	A/G	intronic	90.6	0.135	0.270	0.234	0.001	Protein enabled homolog
RAB3GAP2	A54841857G	A/G	intronic	94.0	0.375	0.496	0.469	0.341	Rab3 GTPase-activating protein non-catalytic subunit