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Polymorphism Analysis on the Second Intron of the *GH* Gene in Chinese Donkeys

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Abstract: To reveal the genetical diversity of different donkey breeds at molecular level and provid some bases for the breed resource evaluation and utilization of donkeys in China, polymorphism of the second intron of the *GH* gene was analysised by PCR-SSCP in the seven donkey breeds (LX, GZ, XJ, GL, HB, DZ, JN). The results showed three haplotypes with the percentage of 1.7% in 174 samples were obtained, the haplotype diversity was high as 0.678 and 0.542 for the Linxian and Huaibei donkey and low as 0.077 for Xingiiang donkey with that of 0.409 and 0.462 for Guangling donkey and Guanzhong donkey, respectively with that of 0.355 and 0.304 for Dezhou donkeys and Jinnan donkeys, respectively. The amplified fragments of A and B haplotypes were cloned and sequenced. The result showed the fragments of B haplotypes had one substitution mutation at 735 site (G→C), the fragments of A haplotypes had one substitution mutation at 869 site (G→T). The results confirmed that there were polymorphisms in the second intron (214 bp) of donkey *GH* gene for the first time.

Key words: Donkey, *GH* gene, PCR-SSCP, genetics diversity, mutation, polymorphisms

INTRODUCTION

The Growth Hormone (GH) is a single chain polypeptide hormone secreted by the anterior pituitary it plays an important role during animal growth and development, studies have shown that *GH* gene consists of 5 exons and 4 introns in mammals and birds (De Noto *et al*., 1981; Woychik *et al*., 1982; Barta *et al*., 1991; Buggioti and Primmier, 2006) and is located on 19th chromosome in q26-qter band region (Hediger *et al*., 1990).

GH gene is the major gene controlling the level of GH secretion, regulating animal growth and development and other important physiological activities. At present, there are a lot of reports on associations of *GH* gene polymorphism with production traits (Horvat and Medrano, 1995; Yardibi *et al*., 2009; Nielsen *et al*., 1995) but the reports on *GH* gene polymorphism in donkey is seldom.

To reveal the genetical diversity of chinese local donkey breeds at molecular level, the *GH* gene sequences of the second intron in the 174 individuals of seven Chinese local donkey breeds were analysied by PCR-SSCP in this study. In order to provide reference for the protection of local donkey breeds germplasm.

MATERIALS AND METHODS

Genomic DNA extraction: The blood samples of 174 donkeys of 7 local breeds (Table 1) were collected and genomic DNA extracted by applying convention method.

Table 1: The number, collection location and origin of samples of donkey breeds

Breeds	Code	No. of individuals	Collection location	Localities
Guangling	GL	26	Guangling town	Guangling and Lingqiu of Shanxi province
Linxian	LX	8	Linxian Shanxi	Linxian West of Shanxi province
Jinnan	JN	28	Wenxi town	Yuncheng and Linfen of Shanxi province
Guanzhong	GZ	32	Yangling Shaanxi	Guanzhong plain of Shaanxi province
Xinjiang	XJ	26	Yining city Xinjiang	Kashi region, Xinjiang Autonomous region
Dezhou	DZ	36	Lucheng Shanxi	The towns along Bohai sea at Lubei plain in Shandong province
Huaibei	HB	18	Mengcheng Anhui	Huaibei city, Anhui province

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PCR amplification: Through using Primer3.0, the second intron primers of *GH* gene were designed according to the DNA sequence of Chinese dwarf horse (DQ845298) and Thoroughbred (DQ845297), the upstream primer is 5'-CT GGCTGCTGACACCTACAA-3', the downstream primer is 5'-CGCTCCTGGGAGAAAGAAC-3'.

The PCR amplification was carried out in a total volume of 25 uL (10×buffer 2.5 mL, dNTPs 2 uL, mix primer 2 uL, Taq DNA polymerase 0.2 uL, template DNA 2 and 14.8 uL sterilization distilled water). PCR was performed under the following reaction procedure: 94°C denaturation for 2.5 min→32×(94°C 40 sec, 58°C 40 sec, 72°C, 1 min)→72°C extension 10 min.

The genotype identification with PCR-SSCP and sequencing confirmation: The genotype of the product of PCR was identified by SSCP procedure as follows, 3 uL PCR product was mixed with 6 uL loading buffer, heating at 98°C for 10 min then bathing in ice for 10 min and visualizing with 12% non-denatured polyacrylamide gel electrophoresis by the silver nitrate dyeing. The PCR fragment were purified with a DNA Fragment Purification kit (TaKaRa Biotechnolgy Dalian Co., Ltd.) then cloned and sequenced by Shanghai Biology Engineering Technology Ltd. (Beijing Sequencing Department).

Statistical analysis: The number of haplotype frequency and the diversity of haplotype were analysed by Microsoft Excel analysis. The diversity of haplotype was calculated by the equation as follows:

$$H = \frac{n\left(1-\sum x_i^2\right)}{(n-1)}$$

H = Haplotype diversity indices
x_i = Means the haplotype ranks i

RESULTS AND DISCUSSION

Haplotype distribution and frequency: The amplified products of the *GH* gene second intron on 7 breeds were analysed by the SSCP (Fig. 1) there are three kinds of haplotypes were detected in the intron of 214 bp and compared with the results of different haplotype after sequenceing, showed the A-type occur a mutation (G→T) in 869 bp of the donkey *GH* gene, the B-type occur a mutation (G→C) in 735 bp (Fig. 2) and both the mutations are transversions with homology 99%. Table 2 shows the detected haplotype and its frequency in different donkey breeds there 131 haplotype of A was detected in 174 samples, the haplotype of A and its frequence is the most in 7 breeds except LX donkey that showed it is the shared haplotype. The haplotype of B was detecteded in 4 individual from 4 donkey breeds (GZ, LX, XJ and HB) it was small with only one individual detected in each breed. Haplotype C was detected in all the donkey breeds except XJ donkey and its frequency range was 0.179-0.5 in 7 breeds.

Haplotype diversity: Table 3 shows the genetic diversity indices of 7 Chinese local donkeys, the the genetic diversity indices of LX donkey was the highest (0.678) showing its genetic diversity is the most abundant, XJ dinkey is the lowest (0.077) its genetic diversity is lack relative while the genetic diversity indices of GL and GZ is closer, 0.409 and 0.462, respectively which is consistent with the record that Shanxi large-sized donkey breeds are local variety and formed after the Guanzhong donkey distributes to these areas (Zhang and Zhu, 1986).

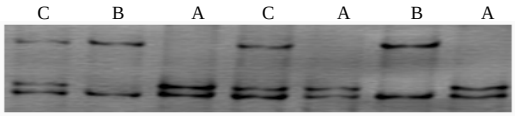

Fig. 1: Detection of SSCP in the intron 2 of the donkey *GH* gene

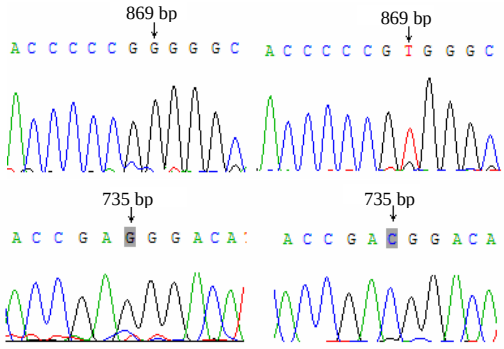

Fig. 2: Chromatograms showing sequence variations in 735 and 869 bp sites of donkey *GH* gene intron 2

Table 2: Haplotype and frequency in the intron of the 7 donkeys *GH* gene

	Breeds							
Haplotype	DZ	GZ	GL	LX	JN	XJ	HB	Total
A	28/0.778	22/0.688	19/0.731	3/0.375	23/0.821	25/0.961	11/0.611	131/0.740
B	-	1/0.031	-	1/0.125	-	1/0.038	1/0.056	4/0.022
C	8/0.222	9/0.281	7/0.269	4/0.500	5/0.179	-	6/0.333	39/0.224
Total	36/1.000	32/1.000	26/1.000	8/1.000	28/1.000	26/1.000	18/1.000	174/1.000

Table 3: Genetic diversity indices in the 2 intron of the 7 donkeys *GH* gene

Breeds	Samples	No. of haplotype	Haplotype proportion	Haplotype diversity
DZ	36	2	0.056	0.355
GZ	32	3	0.093	0.462
GL	26	2	0.077	0.409
LX	8	3	0.375	0.678
JN	28	2	0.071	0.304
XJ	26	3	0.115	0.077
HB	18	3	0.167	0.542

It is clear that the genetic diversity is not significantly in 7 donkey breeds whether it is related to donkey production traits still needs further study.

CONCLUSION

This experiment was conducted to study the polymorphism on intron 2 of *GH* gene in donkey. Three haplotypes were found in 7 China local donkeys but the genetic diversity is not significantly in 7 donkey breeds whether it is associations with donkey production traits still needs further study after expanding number of donkey breeds and samples.

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