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Polymorphism Analysis on Intron 3 of the *GH* Gene in Chinese Donkey

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Abstract: This experiment was conducted to study the polymorphism on intron 3 of *GH* gene in donkey in China. The polymorphism was analysised by PCR-SSCP in the 10 donkey breeds (JN, GL, GZ, DZ, JM, BY, QY, LZ, TH and XJ) by PCR-SSCP. One SNPs was found in the sequence of *GH* gene through the sequencing analysis of the two homozygous genes *AA* and *BB*, the mutation at 100th site (G→T) of the third intron of donkey *GH* gene was showed. Three genotypes AA, AB and BB were detected in 10 donkey breeds and the allele A was the predominant allele. All the breeds were in Hardy-Weinberg equilibrium at this polymorphic site ($p>0.05$). The results confirmed that there were polymorphisms in the intron 3 of *GH* gene.

Key words: Donkey, *GH* gene, intron 3, polymorphism, PCR-SSCP, China

INTRODUCTION

There are rich donkey resources and long history of donkey breeding in China. A large number of donkeys with good quality are widely distributed in China. Along with the enhancement of agriculture mechanization and the improvement of living conditions, the beast of burden withdrew from the agricultural power main force status gradually. Donkey meat is delicious and has high medicinal value (The eguus asinus was maded of donkey skins), it is certain that the donkey will gradually become economic animal used for meat and medicine. The genetic diversities and genetic relationship of Chinese donkey breeds have important guiding sense on researching the donkey origin evolution, conserving the germplasm resources and scientific exploiture.

Growth Hormone (GH) 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) but the report on *GH* gene polymorphism in donkey is seldom. To reveal the genetical diversity of Chinese indigenous donkey breeds at molecular level, the *GH* gene sequences of the intron 3 in the 280 individuals of 10 breeds were analysied by PCR-SSCP in this study. In order to provide reference for conserving the germplasm resources and scientific exploiture of Chinese indigenous donkey breeds.

MATERIALS AND METHODS

DNA samples: The blood samples of 280 donkeys of 10 local breeds (Table 1) were collected and genomic DNA extracted by Applying Convention Method (Sambrook *et al*., 1989).

Primers design: The third intron primers of *GH* gene were designed according to the DNA sequence (DQ845298 and DQ845297), Using Primer 3.0, the upstream primer is 5'-GATGAGGCCCAGCAGAGAT-3', the downstream primer is 5'-GAGCAGCTCCATGTCCTG-3'.

PCR amplification: 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

Table 1: Sample size, genotype and gene frequency in 10 donkey breeds

			Genotype frequency			Allele frequency	
		Sample					
Population	Code	size	AA	AB	BB	A	B
Jinnan	JN	36	27 (0.750)	7 (0.194)	2 (0.056)	0.8472	0.1528
Guangling	GL	32	23 (0.719)	7 (0.219)	2 (0.062)	0.8281	0.1719
Guanzhong	GZ	26	19 (0.731)	5 (0.192)	2 (0.077)	0.8269	0.1731
Dezhou	DZ	28	19 (0.679)	7 (0.250)	2 (0.071)	0.8036	0.1964
Jiami	JM	30	25 (0.833)	4 (0.133)	1 (0.034)	0.9000	0.1000
Biyang	BY	22	19 (0.864)	3 (0.136)	0 (0.000)	0.9318	0.0682
Qingyang	QY	21	17 (0.810)	4 (0.190)	0 (0.000)	0.9048	0.0952
Liangzhou	LZ	30	25 (0.833)	5 (0.167)	0 (0.000)	0.9167	0.0833
Taihang	TH	29	17 (0.586)	8 (0.276)	4 (0.138)	0.7241	0.2759
Xinjiang	XJ	26	15 (0.577)	8 (0.308)	3 (0.115)	0.7308	0.2692

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performed under the following reaction procedure: 94°C denaturation for 10 min→32× (94°C for 40 sec, 56°C for 40 sec and 72°C for 1 min) →72°C extension 10 min.

SNPs 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 population genetic parameters including allele frequency and genotype frequency, Homogeneity (Ho), Heterozygosity (He), effective No. of alleles (Ne) were calculated by Statistical Software POPGENE32 (Yeh *et al*., 1999) Polymorphic Information Content (PIC) values were calculated by the equation as follows:

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

Where:

n = The number of alleles
P_i = The frequency of allele i
P_j = The frequency of allele j

Where PIC is a target for measuring the extent of population polymorphism proposed by Botstein *et al*. (1980) showing highly or lowly polymorphic with a threshold of PIC>0.5 or PIC<0.25, respectively. Effective information content of population and genotype distribution for Hardy-Weinberg equilibrium was tested by POPGENE32.

RESULTS AND DISCUSSION

Polymorphisms analysis: The fragment, about 235 bp for the intron 3 of *GH* gene in Chinese indigenous donkey breeds was successfully amplified (Fig. 1). By applying the PCR-SSCP Method, three kinds of genotype, AA, AB and BB were detected (Fig. 2).

Sequencing of SSCP fragment of *GH* gene: Compared with the results of different genetype after sequenceing,

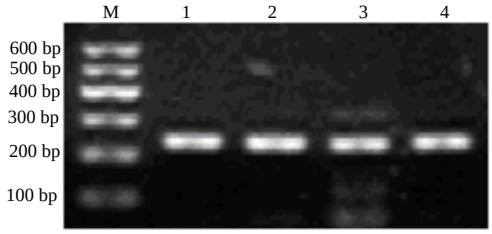

Fig. 1: PCR amplification of *GH* gene; M: Molecular weight marker

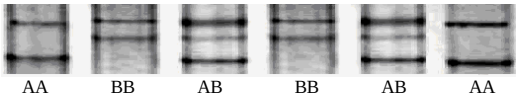

Fig. 2: Detection of SSCP in the intron 2 of the donkey *GH* gene

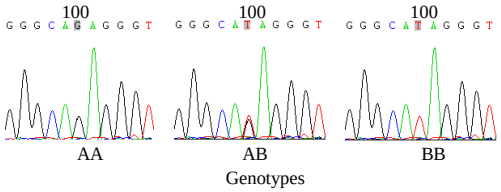

Fig. 3: Chromatograms showing sequence variations in 100 bp sites of donkey *GH* gene intron 3

showed the have a mutation of G→T in 100 bp of the third intron of donkey *GH* gene and forming the two alleles A and B the three genotypes AA, AB and BB (Fig. 3).

Population genetic analysis: For the third intron of donkey *GH* gene in Chinese indigenous breeds, researchers detected their genotypes and calculated the corresponding genotype frequencys, gene frequencys, Ho, He, Ne and PIC (Table 1 and 2). In Table 1, three genotype (AA, AB and BB) were detected in JN, GL, GZ, DZ, JM, TH and XJ only two genotype (AA, AB) were detected in BY, QY, LZ and genotypes of AA express a great advantage as well as the frequency of allele A. In Table 2, the allele distribution of the intron 3 of *GH* gene in all the experimental breeds were in agreement with Hardy-Weinberg equilibrium by the χ^2-test ($p>0.05$), the result indicate a genetic dominance in adaptability and an equilibrium state after a long evolution and selection. The heterozygosity in the descending order was 0.3077, 0.2759, 0.2500, 0.2188, 0.1944, 0.1923, 0.1905, 0.1667, 0.1364 and 0.1333, respectively in the XJ, TH, DZ, GL, JN, GZ, QY, LZ, BY and JM.

Polymorphic information content in the descending order was 0.3197, 0.3161, 0.2658, 0.2453, 0.2442, 0.2254, 0.1638, 0.1574, 0.1411 and 0.1190, respectively in the TH,

Table 2: Population genetic parameter, Hardy-Weinberg equilibrium analysis in 10 donkey breeds

Population	Ho	He	Ne	PIC	χ^2-test	p-vaule
JN	0.8056	0.1944	1.3493	0.2254	2.6320	0.1047
GL	0.7812	0.2188	1.3980	0.2442	2.0619	0.1510
GZ	0.8077	0.1923	1.4010	0.2453	3.3499	0.0672
DZ	0.7500	0.2500	1.4613	0.2658	1.5000	0.2207
JM	0.8667	0.1333	1.2195	0.1638	2.6156	0.1058
BY	0.8636	0.1364	1.1456	0.1190	0.0768	0.7816
QY	0.8095	0.1905	1.2082	0.1574	0.1707	0.6795
LZ	0.8333	0.1667	1.1803	0.1411	0.1953	0.6586
TH	0.7241	0.2759	1.6653	0.3197	3.1610	0.0754
XJ	0.6923	0.3077	1.6488	0.3161	1.5022	0.2203

XJ, DZ, GZ, GL, JN, JM, QY, LZ and BY. The results show a low polymorphism reasons may caused by the number of donkey raised reduced gradually, inbreeding within populations making the continued purification for favorable genes as well as some alleles lost, resulting in the reduction of its PIC. It suggested to us that we should protect the germplasm resources of donkey. The other reason, low PIC vaules may also be less samples in the test population.

CONCLUSION

This experiment was conducted to study the the polymorphism on the intron 3 of *GH* gene in donkey. Three genotypes were found in 10 China local donkeys. All the populations were in Hardy-Weinberg equilibrium at the third intron of the polymorphic site ($p>0.05$). The results confirmed that there were polymorphisms in the intron 3 of *GH* gene whether the polymorphisms is associations with donkey production traits still needs further study.

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