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# Polymorphisms in the Third Exon of *GH* Gene in Chinese Indigenous Donkey

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**Abstract:** To reveal genetic diversity for the breed resource evaluation and utilization of Chinese indigenous donkey, polymorphisms in the third exon of *GH* gene was analyzed by PCR-SSCP and DNA sequencing methods in 16 donkey breeds (DZ, GZ, GL, JN, BH, LX, JM, QY, BY, HY, LZ, HB, GY, YY, TH and XJ). The results showed that there was a transition at nucleotide position 957 genomic DNA of *GH* gene, named as GH-exon3-G957A which led to a glutamic acid to lysine substitution at amino acid position 81. Two different alleles, A and B were identified and three genotypes were observed, AA, AB and BB with the frequency distribution of allele A from 0.6667-0.8333 in the analyzed populations. The genetic diversity analysis revealed that PIC values were between 0.346 and 0.491 except JM donkey (0.239), implying that this locus within *GH* gene possessed a moderate genetic diversity in Chinese indigenous donkeys. The $\chi^2$-test showed that LX, GY and TH donkeys were no significant deviation, GZ, JN, QY, HB and XJ donkey were significant deviation, DZ, GL, BH, JM, BY, HY, LZ and YY donkey were very significant deviation. The results confirmed that there were polymorphisms in the third exon of *GH* gene for the first time.

**Key words:** Donkey, *GH* gene, exon 3, polymorphism, PCR-SSCP, glutamic acid

## INTRODUCTION

Growth Hormone (GH) is a peptide hormone with about 190 residues which regulates growth, development and various metabolic activities (Sterle *et al*., 1995; Ran *et al*., 2004). The *GH* gene belongs to a gene family that also includes the chorionic somatomammotropin (placental lactogen) gene, the prolactin gene and several prolactin-like genes, 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). There are abundant polymorphisms in domestic animal *GH* gene and it associated with many quantitative trait, growth rate, milk production rate, milk fat percentage (Falaki *et al*., 1997; Lee *et al*., 1996; Marques *et al*., 2003; Zhou *et al*., 2005) and reproductive performance (Lechniak *et al*., 1999) while in donkey, the growth hormone gene sequences was cloned successfully in 2011 (Zhu *et al*., 2006) but the study on polymorphisms was seldom.

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 made of donkey skins), it is certain that the donkey will gradually become economic animal used for meat and medicine. Therefore, the DNA polymorphisms in exon 3 of *GH* gene in the 428 individuals of 16 breeds were analyzed by PCR-SSCP and sequencing in this study. To reveal the genetical diversity in Chinese indigenous donkey breeds at molecular level.

## MATERIALS AND METHODS

**Specimen collection and DAN extraction:** About 428 blood samples of 16 indigenous donkey breeds were collected from conservation farms or origin location, respectively; Dezhou donkey (DZ, N = 36), Guanzhong donkey (GZ, N = 32), Guangling donkey (GL, N = 26), Jinnan donkey (JN, N = 28), Bohai donkey (BH, N = 27), Lixin donkey (LX, N = 10), Jiami donkey (JM, N = 30), Qingyang donkey (QY, N = 22), Biyang donkey (BY, N = 21), Huaiyang donkey (HY, N = 20), Liangzhou donkey (LZ, N = 27), Huaibei donkey (HB, N = 18), Guyuan donkey (GY, N = 20), Yuangyuan donkey (YY,

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N = 35), Taihang donkey (TH, N = 29) and Xinjiang donkey (XJ, N = 26). DNA was isolated from the blood and extracted by phenol/chloroform mixture.

**Amplification of the exon 3 of *GH* gene:** The DNA amplification of the *GH* gene was achieved by PCR. A pair PCR primer, the upstream primer (5'-AACCGTGCACCAGCTTAGAC-3') and the downstream primer (5'-GCAGGGCCACTCACAGAT-3') was designed according to the DNA sequence (DQ845298 and DQ845297). The PCRs were carried out in 25 μL volumes (10×buffer 2.5 mL, dNTPs 2 μL, mix Primer 2 μL, Taq DNA polymerase 0.2 μL, template DNA 2 μL and 14.8 μL sterilization distilled water). The thermal profile consisted of 10 min at 94°C followed by 32 cycles of 40 sec at 94°C, 40 sec at 58°C and 40 sec at 72°C with a final extension of 10 min at 72°C. Amplification was carried out in Mastercycler (Eppendorf, Germany).

**Single Strand Confirmation Polymorphism (SSCP):** PCR products were mixed with 6 μL of denaturing loading dye [95% (w/v) deionized formamide, 0.05% (w/v) xylene cyanol, 0.05% (w/v) bromophenol blue and 0.02M EDTA] in a total volume of 9 μL. The mixture was denatured at 95°C for 10 min and was snap chilled on ice (Pipalia *et al*., 2004). The total volume was applied in a 12% polyacrylamide gel as described by Herring *et al*. (1982). The electrophoresis was performed in 0.5X TBE buffer (Tris 100 mM, boric acid 9 mM, EDTA 1 mM) at room temperature (18°C) and constant 160 V for 12 h. Polyacrylamide gels were stained with silver according to the protocol described (Herring *et al*., 1982).

**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 = Number of alleles
$P_i$ = Frequency of allele i
$P_j$ = 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

**PCR-SSCP analysis of *GH* gene:** All extracted DNAs from donkey blood samples yielded a specific single band PCR product without any nonspecific band. Therefore, the PCR products were directly used for SSCP analysis. The allelic variation in the *GH* gene was examined by PCR-SSCP. The non-denaturing gel electrophoresis enabled the visualization of ssDNA and was analyzed for SSCP band patterns. In the study, a total of three SSCP patterns were observed in the examined donkey (Fig. 1).

**Sequencing of SSCP fragment of *GH* gene:** Compared with the results of different genotype after sequencing, showed there have a transition mutation of G→A in 957 bp of the third exon of donkey *GH* gene, named as GH-exon3-G957A which led to a conservative Glutamic acid to Lysine substitution at amino acid position 81 and forming the two alleles A and B, the three genotypes AA, AB and BB (Fig. 2).

**Population genetic analysis:** For the third exon of donkey *GH* gene in Chinese indigenous breeds, researchers detected their genotypes and calculated the corresponding genotype and allelic frequencies, Ho, He, Ne and PIC (Table 1 and 2). In Table 1, three genotypes (AA, AB and BB) were detected in analyzed populations and genotypes of AA expressed a great advantage as well as the frequency of allele A. The allele distribution of SNP in the third exon of *GH* gene in LX, GY and TH donkeys

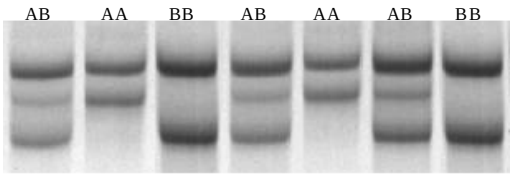


Fig. 1: SSCP polymorphism of Chinese indigenous donkey *GH* gene. Three different PCR-SSCP patterns (genotype) were identified

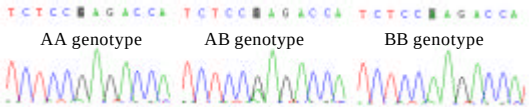


Fig. 2: The sequencing and sequence comparison results of donkey *GH* gene and SNPs are indicated by shaded sections

Table 1: Genotype and allele frequencies, Hardy-Weinberg equilibrium analysis in 16 donkey breeds

| | Genotype frequency | | | Allele frequency | | Chi-square test | |
|---|---|---|---|---|---|---|---|
| Population | AA | AB | BB | A | B | $\chi^2$ | p-vaule |
| DZ | 0.6316 | 0.1842 | 0.1842 | 0.7639 | 0.2361 | 8.3117 | 0.004 |
| GZ | 0.7187 | 0.1875 | 0.0938 | 0.8125 | 0.1875 | 5.3590 | 0.021 |
| GL | 0.6563 | 0.1875 | 0.1562 | 0.7500 | 0.2500 | 8.7084 | 0.003 |
| JN | 0.6487 | 0.2162 | 0.1351 | 0.7568 | 0.2432 | 6.8669 | 0.008 |
| BH | 0.6666 | 0.1481 | 0.1853 | 0.7407 | 0.2593 | 11.0901 | 0.001 |
| LX | 0.7000 | 0.2000 | 0.1000 | 0.8000 | 0.2000 | 2.1125 | 0.146 |
| JM | 0.8333 | 0.0000 | 0.1667 | 0.8333 | 0.1667 | 32.8798 | 0.000 |
| QY | 0.6818 | 0.1818 | 0.1364 | 0.7727 | 0.2273 | 5.0532 | 0.015 |
| BY | 0.6667 | 0.0000 | 0.3333 | 0.6667 | 0.3333 | 22.3362 | 0.000 |
| HY | 0.7000 | 0.0500 | 0.2500 | 0.7250 | 0.2750 | 16.6771 | 0.000 |
| LZ | 0.7000 | 0.0333 | 0.2667 | 0.7167 | 0.2833 | 26.6594 | 0.000 |
| HB | 0.6111 | 0.1667 | 0.2222 | 0.6944 | 0.3056 | 7.4439 | 0.006 |
| GY | 0.6000 | 0.2500 | 0.1500 | 0.7250 | 0.2750 | 3.2708 | 0.071 |
| YY | 0.6571 | 0.1143 | 0.2286 | 0.7143 | 0.2857 | 19.1428 | 0.000 |
| TH | 0.7000 | 0.2333 | 0.6667 | 0.8167 | 0.1833 | 1.7795 | 0.1822 |
| XJ | 0.6538 | 0.1923 | 0.1539 | 0.7500 | 0.2500 | 6.8670 | 0.0088 |

Table 2: Population genetic parameter analysis in 16 donkey breeds

| Population | Ho | He | Ne | PIC |
|---|---|---|---|---|
| DZ | 0.8056 | 0.1944 | 0.3607 | 0.477 |
| GZ | 0.8125 | 0.1875 | 0.3047 | 0.393 |
| GL | 0.8125 | 0.1875 | 0.3750 | 0.457 |
| JN | 0.7838 | 0.2162 | 0.3682 | 0.457 |
| BH | 0.8519 | 0.1481 | 0.3941 | 0.448 |
| LX | 0.8000 | 0.2000 | 0.3200 | 0.410 |
| JM | 1.0000 | 0.0000 | 0.2778 | 0.239 |
| QY | 0.8182 | 0.1818 | 0.3512 | 0.434 |
| BY | 1.0000 | 0.0000 | 0.4444 | 0.346 |
| HY | 0.9500 | 0.0500 | 0.3987 | 0.381 |
| LZ | 0.9667 | 0.0333 | 0.4061 | 0.367 |
| HB | 0.8333 | 0.1667 | 0.4244 | 0.489 |
| GY | 0.7500 | 0.2500 | 0.3987 | 0.491 |
| YY | 0.8857 | 0.1143 | 0.4082 | 0.445 |
| TH | 0.7667 | 0.2333 | 0.2994 | 0.393 |
| XJ | 0.8077 | 0.1923 | 0.3750 | 0.458 |

did not deviated from HWE ($p>0.05$) but it was deviated from HWE at $p<0.05$ in GZ, JN, QY, HB and XJ donkeys and or at $p<0.01$ in DZ, GL, BH, JM, BY, HY, LZ and YY donkeys. These result indicated that LX, GY and TH donkey reached the equilibrium state, GZ, JN, QY, HB, XJ, DZ, GL, BH, JM, BY, HY, LZ and YY donkey did not reached the equilibrium state. The reason may be the number of these donkey were raised decrease or the number of the sample was less.

**Polymorphism of the third exon *GH* gene:** *GH* gene is an attractive candidate gene for livestock production, the polymorphisms identified and its polymorphisms correlating product traits have had many studies in livestock. For example, extensive findings of *GH* gene that associated with milk yield, milk compositions in cattle (Falaki *et al*., 1997; Lee *et al*., 1996; Lagziel *et al*., 1996; Hallerman *et al*., 1987). Associations between GH genetic polymorphisms and variation in growth and fatness traits have been reported in pigs (Nielson and Larsen, 1991; Kirkpatrick, 1992; Larsen and Nielson, 1993). However, to date , the study on donkey *GH* gene polymorphism at home and abroad is rarer. This study presents the first find the third exon of donkey growth hormone gene contains polymorphism however, the polymorphism differences among large, medium and small-sized of donkeys is not significantly whether its polymorphism is related to donkey production traits still needs further study. In order to seek for economic traits genetic linkage marker in livestock, the candidate gene approach is very effective, it may be used to directly study the relationship between gene polymorphism and the individual economic traits. Therefore, it has important theoretical and practical significance, to analyse relationship the donkey *GH* gene mutation and economic traits, further improve the production performance and promote industry development of donkey.

**The genetic diversity of donkey:** In the study, the third exon of *GH* gene polymorphism of 16 donkey breeds were analyzed, the results shows that PIC almost belong to a median polymorphism, it reflected that there was a moderate genetic diversity within the gene in analyzed populations but it is not consistent with the PIC belong to a high polymorphism, analyzed in the same breeds using microsatellites (Zhu *et al*., 2006; Aji *et al*., 2007). The reasons may caused by different detection methods and different detection sites of DNA. The purpose of the research was to provide reference for further study genetic polymorphism and varietal characteristic of Chinese indigenous donkey.

## CONCLUSION

The experiment was conducted to study the polymorphism on the third exon of *GH* gene in donkey. A SNPs, GH-exon3-G957A at nucleotide position 957 of the gene was found, it led to a conservative Glutamic acid to Lysine substitution at amino acid position 81. The PIC in the 16 population analyzed was at median polymorphic level whether the polymorphisms is associations with donkey production traits still needs further study.

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