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Allelic Polymorphism Identified and Analysis on the Fifth Exon of Chinese Indigenous Donkey *GH* Gene by PCR-SSCP

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Abstract: Growth of animal is largely regulated by Growth Hormone (GH). In this study the objective was to investigate variations in the fifth exon of *GH* gene and their polymorphism in 5 Chinese indigenous donkeys by PCR-SSCP and DNA Sequencing Methods. The results showed that there was a transversion at nucleotide position 1802 of *GH* gene in the 5th exon named as GH-exon5-G1802C which led to a conservative Lysine to Asparagine substitution at amino acid position 205. In detail, two different alleles, A and B were identified and three genotypes were observed, AA, AB and BB with the frequency distribution of allele B from 0.5962-0.7344 in analyzed populations. The genetic diversity analysis revealed that all PIC values were between 0.3140 and 0.3656, implying that this locus within *GH* gene possessed moderate genetic diversity in 5 Chinese indigenous donkeys. The χ^2-test showed that GZ donkey was significant deviation, DZ donkey was no significant deviation from Hardy-Weinberg equilibrium, GL, JN and XJ donkey was not in agreement Hardy-Weinberg equilibrium. The results confirmed that there were polymorphisms in the exon 5 of *GH* gene.

Key words: Donkey, *GH* gene, exon3, polymorphism, PCR-SSCP

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). These activities resulting from GH involve binding to its receptor, a member of the cytokine receptor superfamily. Receptor dimerization initiates signal transduction which leads to activation of various intracellular pathways, particularly the non-receptor tyrosine kinase, Jak2 (Waters *et al*., 2006). In all mammals, the *GH* gene extends over 2-3 kb and comprises five exons split by four introns (De Noto *et al*., 1981; Woychik *et al*., 1982; Barta *et al*., 1991). In higher primates, the *GH* gene has expanded by a series of gene duplications to give a gene cluster (MacLeod *et al*., 1992; Golos *et al*., 1993). The porcine Growth Hormone (*pGH*) gene has been provisionally assigned to chromosome 12 band pl.2-1.5 (Yerle *et al*., 1993; Cheng *et al*., 2000). It appears to be a single copy gene. The coding region consists of five exons, covering a total transcribed area of about 1.7 kb (Vize and Wells, 1987). The complete cDNA of horse *GH* gene was known (Ascacio-Martinez and Barrera-Saldana, 1994). The donkey *GH* gene sequence also was reported (Zhu *et al*., 2011) but the study on polymorphism in donkey *GH* gene was seldom. To reveal the genetical diversity of Chinese indigenous donkey breeds at molecular level, the *GH* gene sequences of the fifth exon 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

Specimen collection and DAN extraction: About 163 blood samples of five indigenous donkey breeds were collected from conservation farms or origin location, respectively; Jinnan donkey (JN, N = 36), Guangling donkey (GL, N = 32), Guanzhong donkey (GZ, N = 26), Dezhou donkey (DZ, N = 28) and Xinjiang donkey (XJ, N = 26). DNA was isolated from the blood and extracted by phenol/chloroform mixture (Sambrook *et al*., 1989).

Amplification of the intron 3 of *GH* gene: The DNA amplification of the *GH* gene was achieved by PCR. A pair PCR primer, the upstream primer 5'- CTGGGCA GATCCTCAAGC-3') and the downstream primer (5'-TATT AGGAAAGATGGGTAGGCACT-3'), targeting a fragment

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of 260 bp was employed as described (Zhu *et al*., 2011). The PCRs were carried out in 25 μL volumes (10×buffer 2.5 mL, dNTPs 2 uL, mix Primer 2 uL, Taq DNA polymerase 0.2 uL, template DNA 2 uL and 14.8 uL 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 56°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 0.02 M 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) and Polymorphic Information Content (PIC) values were calculated by the equation as follows:

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

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

PCR-SSCP analysis of *GH* gene: All extracted DNAs from sheep 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 this 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 genetype after sequenceing, showed the have a mutation of G→C in 1802 bp of the fifth exon of donkey *GH* gene, named as GH-exon5-G1802C which led to a conservative Lysine to Asparagine substitution at amino acid position 205 and forming the two alleles A and B, the three genotypes AA, AB and BB (Fig. 2).

Population genetic analysis: For the fifth exon of donkey *GH* gene in Chinese indigenous breeds, researchers detected their genotypes and calculated the corresponding genotype frequencies, gene frequencies, Ho, He, Ne and PIC (Table 1 and 2). In Table 1, three genotype (AA, AB and BB) were detected in analyzed

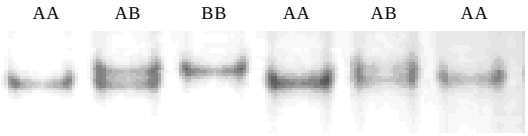

Fig. 1: SSCP polymorphism of Chinese indigenous donkey *GH* gene. Three different PCR-SSCP patterns (genotype) were identified

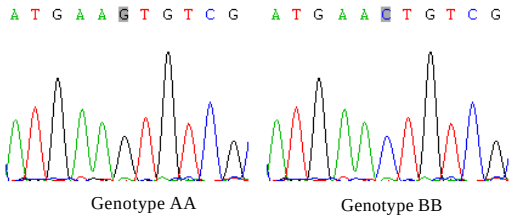

Fig. 2: The sequencing and sequence comparison results of donkey *GH* gene and SNPs are indicated by shaded sections

Table 1: Genotype and allele frequencies, Hardy-Weinberg equilibrium analysis in 5 donkey breeds

	Genotype frequency			Allele frequency		χ^2-test	
Population	AA	AB	BB	A	B	χ^2	p-vaules
JN	0.1945	0.1944	0.6111	0.2917	0.7083	10.7672	0.0010
GL	0.1562	0.2188	0.6250	0.2656	0.7344	6.7562	0.0093
GZ	0.1250	0.3438	0.5312	0.2969	0.7031	1.2014	0.2730
DZ	0.1351	0.2703	0.5946	0.2703	0.7297	4.0549	0.0440
XJ	0.3462	0.1154	0.5384	0.4038	0.5962	15.8733	0.0001

Table 2: Population genetic parameter analysis in 10 donkey breeds

Population	Ho	He	Ne	PIC
JN	0.8056	0.1944	1.7041	0.3278
GL	0.7812	0.2188	1.6397	0.3140
GZ	0.6562	0.3438	1.7167	0.3303
DZ	0.7297	0.2703	1.6514	0.3167
XJ	0.8846	0.1154	1.9287	0.3656

populations and genotypes of BB express a great advantage as well as the frequency of allele B. The allele distribution of the fifth exon of *GH* gene in GZ donkey was significant deviation ($p>0.05$), DZ donkey was no significant deviation ($p>0.01$) from Hardy-Weinberg equilibrium, GL, JN and XJ donkey was not in agreement Hardy-Weinberg equilibrium ($p<0.01$). These result indicate that GZ donkey was at equilibrium state after a long evolution and selection, DZ, GL, JN and XJ donkey may be artificial selection in breeding. In Table 2, the heterozygosity in the descending order was 0.3438, 0.2703, 0.2188, 0.1944 and 0.1154, respectively in the GZ, DZ, GL, JN and XJ. Polymorphic information content in the descending order was 0.3656, 0.3303, 0.3278, 0.3167 and 0.3140, respectively in the XJ, GZ, JN, DZ and GL.

The results show a median polymorphism, it reflected that there was a moderate genetic diversity within the gene in analyzed populations, 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 researchers 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 fifth exon of *GH* gene in donkey. A SNPs, GH-exon5-G1802C was found, it led to a conservative Lysine to Asparagine substitution at amino acid position 205. The PIC in the five population analyzed was at median polymorphic level whether the polymorphisms is associations with donkey production traits still needs further study.

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REFERENCES

Ascacio-Martinez, J.A. and H.A. Barrera-Saldana, 1994. Sequence of cDNA encoding horse growth hormone. Gene, 143: 299-300.

Barta, A., R.I. Richards and J.D. Baxter, 1991. Primary structure and evaluation of rat growth hormone gene. Proc. Natl. Acad. Sci., 78: 4867-4871.

Botstein, D., R.L. With, M. Skolnick and R.W. Davis, 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Cheng, W.T.K., C.H. Lee, C.M. Hung, T.J. Chang and C.M. Chen, 2000. Growth hormone gene polymorphisms and growth performance traits in Duroc, Landrace and Tao-Yuan pigs. Theriogenology, 54: 1225-1237.

De Noto, F.M., D.D. Moore and H.M. Goodman, 1981. Human growth DNA sequence and mRNA structure: Possible alternative splicing. Nucleic Acids Res., 97: 3719-3730.

Golos, T.G., M. Durning, J.M. Fisher and P.D. Fowler, 1993. Cloning of four growth hormone/chorionic somatomammotropin-related complementary deoxyribonucleic acids differentially expressed during pregnancy in the rhesus monkey placenta. Endocrinology, 133: 1744-1752.

Herring, A.J., N.F. Inglis, C.K. Ojeh, D.R. Snodgrass and J.D. Menzies, 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver stained polyacrylamide gels. J. Clin. Microbiol., 16: 473-477.

MacLeod, J.N., A.K. Lee, S.A. Liebhaber and N.E. Cooke, 1992. Developmental control and alternative splicing of the placentally expressed transcripts from the human growth hormone gene cluster. J. Biol. Chem., 267: 14219-14226.

Pipalia, D.L., C.G. Joshi, D.N. Rank, B.P. Brahmkshtri and J.V. Solanki, 2004. PCR-SSCP typing of MHC in cattle and buffaloes. Ind. J. Anim. Sci., 74: 637-639.

Ran, X.Q., W.S. Li and H.R. Lin, 2004. Stimulatory effects of gonadotropinreleasing hormone and dopamine on growth hormone release and growth hormone mRNA expression in *Epinephelus coioides*. Acta Physiol. Sin., 56: 644-650.

Sambrook J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, New York, USA.

Sterle, J.A., T.C. Cantley, W.R. Lamberson, M.C. Lucy, D.E. Gerrard, R.L. Matteri and B.N. Day, 1995. Effects of recombinant porcine somatotropin on placental size, fetal growth and IGF-I and IGF-II concentrations in pigs. J. Anim. Sci., 73: 2980-2985.

Vize, P.D. and J.R. Wells, 1987. Isolation and characterization of the porcine growth hormone gene. Gene, 55: 339-344.

Waters, M.J., H.N. Hoang, D.P. Fairlie, R.A. Pelekanos and R.J. Brown, 2006. New insights into growth hormone action. J. Mol. Endocrinol., 36: 1-7.

Woychik, R.P., S.A. Camper, R.H. Lyons, S. Horowitz and E.C. Goodwin *et al*., 1982. Cloning and nucleotide sequencing of the bovine growth hormone gene. Nucleic Acids Res., 10: 7197-7210.

Yeh, F.C., T.J.B. Boyle, Y.Z. Ye and J.M. Xiyan, 1999. Popgene version 1.32. Microsoft window-based freeware for population genetics analysis. University of Alberta and Center for International Forestry Research, Canada.

Yerle, M., Y. Lahbib-Mansais, P.D. Thomsen and J. Gellin, 1993. Localization of the porcine growth hormone gene to chromosome 12p1.2-->p1.5. Anim. Genet., 24: 129-131.

Zhu, W.J., J. Chen, X.M. Guan, J.H. Wu and L.Y. Geng *et al*., 2011. Cloning and sequence analysis of growth hormone gene from donkey. Scientia Agric. Sinica, 44: 579-586.