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POLYMORPHISMS OF EXON 5, EXON 7 AND INTRON 10 OF MMP2 GENE AND THEIR ASSOCIATION WITH WOOL DENSITY IN REX RABBITS

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Abstract: Wool density is an important index that influences Rex rabbit fur quality. In our earlier studies, we found some important differentially expressed genes in different wool density of Rex rabbit by cDNA microarray. Based on the outcome, we conducted an association study to identify single nucleotide polymorphisms (SNPs) of exon 1, 5, 7 and 10 of matrix metalloproteinase-2 (MMP2) gene and their ligands associated with wool density. The results showed that exon 1 and exon 10 of MMP2 gene did not occur mutation in 100 Rex rabbits, meanwhile 3 SNPs were identified in exon 5, exon 7 and intron 10 of MMP2 gene sequence respectively, the 3 mutation sites were as follows: MMP2-exon 5-26C/G, MMP2-exon 7-101C/T and MMP2-intron 10-6C/T. The 3 SNPs were all in Hardy-Weinberg equilibrium. Phenotypic correlation analysis results showed the 3 mutations lacked significant associations (P>0.05) with the wool density.

Key Words: Rex rabbit, MMP2 gene, SNPs, fur, wool density.

INTRODUCTION

As Rex rabbit is a typical rabbit for fur, wool density is one of the most important traits used to evaluate the quality of Rex rabbit. In our earlier studies, gene expression patterns in different wool density of Rex rabbit was identified by cDNA microarray. Some important differentially expressed genes in different wool density of Rex rabbit were identified, such as MMP2, TGF-β1, TGF-β2, IGF-1, BMP2 and CCNA2. The abnormal expression of these genes may play an important role in the initiation and development of hair follicles finally leading to the distinction in wool density of Rex rabbit (Chen *et al.,* 2011a). We conducted an association study to identify single nucleotide polymorphisms (SNPs) within CCNA2 gene and their ligands associated with wool density. Finally, we identified 2 SNPs (129G>A and 1140G>C) in the CCNA2 gene and found that the 2 SNPs have potential effects on wool density in Rex rabbit (Chen *et al.,* 2011b).

Matrix metalloproteinases (MMPs) comprise a family of at least 25 secreted zinc proteinases, which are of great importance not only for the extracellular matrix (ECM) turnover, but also for interactions between cells and their surrounding (Sternlicht *et al.,* 2001; Nagase *et al.,* 2006). Matrix metalloproteinase-2 (MMP2) is an enzyme that degrades components of the extracellular matrix and thus plays a pivotal role in cell migration during physiological and pathological processes (i.e., embryogenesis, tissue remodelling, wound healing and angiogenesis) (Pirilä *et al.,* 2002; Sotnikova *et al.,* 2010; Volcik *et al.,* 2010). No one has done the relevant research on whether the hair coat growth is regulated by MMP2 gene.

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Table 1: Primer sequence, amplified region and product sizes.

Amplified region	Primer sequence (bp)	Product size (bp)
Exon 1	F: AGTCTAGGCAATCGAAAGAGCGGACC	338
	R: TCGGAAAGTTCGACTCAACTTGGCTC	
Exon 5	F: GCACCTCTGTCTACATCGGTGGGATC	375
	R: GGGGTATAGTGGGAAACTCAACAAGGCT	
Exon 7	F: GCACAGGGTTACTGGGTGTTGAAGGG	379
	R: GAGGTCTCCAGCTCTGGCGAGCATTT	
Exon 10	F: TTGGTCTGATTTATTTCATTTCCACTCGT	400
	R: CAGTCTCCCAGGTGACGCCCATGTTT	

In our earlier studies, we found that MMP2 gene expression is up-regulated in the group of Rex rabbits with thick coat (Chen *et al.,* 2011). In this study, we tried to identify SNPs of the Rex rabbit MMP2 gene and determine the association of these SNPs with wool density.

MATERIALS AND METHODS

Animals and DNA samples

This experiment was carried out at a Rex rabbit farm, Baoding, China. The rabbits were housed individually in closed buildings with natural ventilation and had free access to water. The wool density data (Gu *et al.,* 1999) were measured at 22 wk of age. The blood samples were collected from 100 Rex rabbits.

Approximately 5 mL blood per Rex rabbit was taken aseptically from the jugular vein and kept in a tube containing anticoagulant EDTA·Na_2. All samples were delivered back to the laboratory on ice. The genomic DNA was extracted from white blood cells using standard phenol-chloroform extraction protocol.

PCR conditions and DNA sequencing

According to the sequence of the *Oryctolagus cuniculus* MMP2 gene (GenBank accession No. NM_001082209.1, NW_003159251.1), 4 pairs of primer (Table 1) were designed to amplify the exon 1, 5, 7 and 10 of MMP2 gene. Polymerase chain reaction (PCR) was performed in a 50 µL reaction mixture containing 50 ng DNA, 10 pM of each primer, 0.2 mM dNTP, 2.5 mM $MgSO_4$ and 1 U Taq DNA polymerase (Invitrogen, USA). The cycling protocol: 95°C 5 min; 35 cycles of 95°C 30 s, 56°C 30 s, 68°C 30 s; 68°C 10 min. The PCR products were detected by 1.2% agarose gel electrophoresis and purified with the Agarose Gel DNA Purification Kit (TaKaRa, China). The PCR products

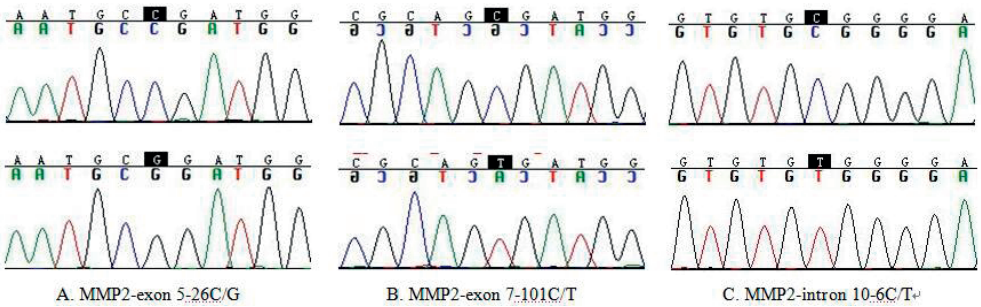

Figure 1: DNA sequencing tracing of the matrix metalloproteinase-2 (MMP2) gene.

were sent to sequence in both directions in an ABI PRIZM 377 DNA sequencer (Perkin-Elmer, USA). Sequences were analysed using DNAStar software to find the SNP.

Statistical analysis

Allele and genotype frequencies and their accordance with the Hardy-Weinberg law were determined by PopGene software version 1.32 (*http://www.ualberta.ca/~fyeh/index.htm*). The association between the polymorphism of the MMP2 gene and wool density was analysed with the GLM procedure of SPSS software version 13.0 (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

Polymorphisms in Rex rabbit MMP2 gene

The polymorphisms of MMP2 gene were detected by DNA sequencing, and we found the exon 1 and exon 10 of MMP2 gene did not cause mutation in the 100 Rex rabbits. Meanwhile, 3 SNPs were identified in exon 5, exon 7 and intron 10 of MMP2 gene sequence, respectively. The 3 mutation sites were as follows: MMP2-exon 5-26C/G, MMP2-exon 7-101C/T and MMP2-intron 10-6C/T. In the original sequences of the mutant region there were no restriction sites, and the base mutations did not create new restriction sites. Example sequencing traces are shown in Figure 1.

The allele and genotype frequencies of the MMP2 gene polymorphisms are presented in Table 2. The observed genotype frequencies were in Hardy-Weinberg equilibrium in the whole sample.

SNP marker association

The genotype of 100 Rex rabbit individuals was compared with the wool density data. The results showed that the 3 mutations at MMP2-exon 5-26C/G, MMP2-exon 7-101C/T and MMP2-intron 10-6C/T lacked any significant associations ($P>0.05$) with wool density. The results of association analysis are depicted in Table 3.

CONCLUSIONS

In conclusion, this paper reports for the first time the role of MMP2 gene polymorphisms in wool density. Three SNPs were identified via sequencing, and although the study suggests no relation between exon 5, 7 and intron 10 of MMP2 gene and wool density in Rex rabbits, our workgroup will continue to analyse the MMP2 gene's polymorphisms.

Table 2: Allele and genotype frequencies of matrix metalloproteinase-2 (MMP2) gene polymorphisms in the 100 experimental Rex rabbits.

Allele and genotype	Frequency (%)
MMP2-exon 5-26C/G	N =100
Allele	
C	89
G	11
Genotype	
CC	81
CG	16
GG	3
MMP2-exon 7-101C/T	N =100
Allele	
C	47
T	53
Genotype	
CC	3
CT	88
TT	9
MMP2-intron 10-6C/T	N =100
Allele	
C	47
T	53
Genotype	
CC	10
CT	74
TT	16

Table 3: Association of SNPs of the matrix metalloproteinase-2 (MMP2) gene with wool density in Rex rabbit.

Genotype	Wool density (pieces per cm^2)
MMP2-exon 5-26C/G	
CC (81)	15759±1491
CG (16)	15379±1237
GG (3)	15620±1024
MMP2-exon 7-101C/T	
CC (3)	15762±1184
CT (88)	15705±1354
TT (9)	15904±1303
MMP2-intron 10-6C/T	
CC (10)	15596±1246
CT (74)	15669±1383
TT (16)	15861±1107

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REFERENCES

Chen S.J., Liu T., Liu Y.J., Dong B., Gu Z.L. 2011a. Gene expression patterns in different wool densities of Rex rabbit using cDNA microarray. *Agri. Sci. China, 10: 595-601. https://doi.org/10.1016/S1671-2927(11)60041-2*

Chen S.J., Liu T., Liu Y.J., Dong B., Huang Y.T., Gu Z.L. 2011b. Identification of single nucleotide polymorphisms in the CCNA2 gene and its association with wool density in Rex rabbits. *Genet. Mol. Res., 4: 3365-3370. https://doi.org/10.4238/2011.November.4.1*

Sternlicht M.D., Werb Z. 2001. How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell. Dev. Biol., 17: 463-516. https://doi.org/10.1146/annurev.cellbio.17.1.463*

Nagase H., Visse R., Murphy G. 2006. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc. Res., 69: 562-573. https://doi.org/10.1016/j.cardiores.2005.12.002*

Volcik K.A., Campbell S., Chambless L.E., Coresh J., Folsom A.R., Mosley T.H., Ni H., Wagenknecht L.E., Wasserman B.A., Boerwinkle E. 2010. MMP2 genetic variation is associated with measures of fibrous cap thickness, The Atherosclerosis Risk in Communities Carotid MRI Study. *Atheroscler., 1: 188-193. https://doi.org/10.1016/j.atherosclerosis.2009.12.006*

Pirilä E., Parikka M., Ramamurthy N.S., Maisi P., McClain S., Kucine A., Tevarhartiala T., Prikk K., Golub L.M., Salo T., Sorsa T. 2002. Chemically modified tetracycline (CMT- 8) and estrogen promote wound healing in ovariectomized rats: effects on matrix metalloproteinase-2, membrane type 1 matrix metalloproteinase, and laminin-5 γ2-chain. *Wound Repair Regen., 10: 38-51. https://doi.org/10.1046/j.1524-475X.2002.10605.x*

Sotnikova N.Y., Antsiferova Y.S., Posiseeva L.V., Shishkov D.N., Posiseev D.V., Filipova E.S. 2010. Mechanisms regulating invasiveness and growth of endometriosis lesions in rat experimental model and in humans. *Fertil. Steril., 93: 2701-2705. https://doi.org/10.1016/j.fertnstert.2009.11.024*

Gu Z.L., Ren W.S., Huang R.L., Huang Y.T., Chen B.J. 1999. Study on density of rex rabbit. *Chinese J. Rabbit Farming. 4: 18-21. [in Chinese].*