

Detection of 881^A 881^G Mutation in Tyrosinase Gene and Associations with the Black Ear Coat Color in Rabbits

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ABSTRACT : The tyrosinase gene was selected as a candidate for uncovering genetic mechanism causing “black ear” coat color in rabbits. A PCR-SSCP detection method was established for the 881^A 881^G mutation located in the central region of the tyrosinase gene between the CuA and CuB binding region signatures, and this was confirmed by sequencing and alignment. Fully consistent associations between the SNP and “black ear” coat color were observed by analysis in a “black ear” pedigree and on 61 unrelated individuals. This SNP can serve as a molecular marker for use in “black ear” wool rabbit breeding. (*Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 10 : 1395-1397*)

Key Words : Rabbit , Black Ear Coat Color, Tyrosinase Gene, PCR-SSCP, SNP Marker

INTRODUCTION

Coat color is an economically important trait in rabbits, ranging from complete pigmentation of coat and eyes to “albino” type. To develop a high-wool producing “black ear” strain, the *Yipulu* breed which shows pigmentation only at the extremes was selected as one parental strain. Identification of the gene responsible for “black ear” and establishment of an easily operated and effective detection method were essential in rabbit breeding.

In mice and human beings, mutations in the tyrosinase gene were shown to give rise to different pigmentation types (Jackson, 1994; Oetting and King, 1999). Its product, melanin, displayed in the melanocytes of skin and eyes is the cause of different coat colors. The coding sequence of the rabbit tyrosinase gene has a length of 1,593 nt (GenBank Access. No. AF210660) and is composed of five exons. Studies had shown that the 881^A 881^G mutation, which is located in the central region of the tyrosinase gene between the CuA and CuB binding region signatures, is strain-specific for California rabbit for its unique “eight black” coat color at extremes (Aigner et al., 2000). In the present study, the tyrosinase gene was chosen as the candidate to uncover associations with “black ear” coat type as well as to establish an easy method for practical detection.

MATERIALS AND METHODS

Materials

Samples of rabbit ear notch were collected from Shengjing and Niuquan Elite Rabbit Breeding Center of

Laiwu City and stored in 70% ethanol at -20°C. EDTA , xylene cyanol FF, bromophenol blue, and agrose were from Sino-America Biotechnology Ltd . Acrylamide, bis-acrylamide, TEMED, ammonium persulfate, formamide , ethanol , AgNO₃ and citrate sodium were from Beijing Chemical Reagent Company. Proteinase K were from Merck Co Ltd of Germany and dNTPs from Gibco BRL Co Ltd. Taq polymerase, DNA recovery kit for PCR products and pMD18-T vector were from TaKaRa Co Ltd (Dalian).

DNA extraction

Rabbit genomic DNA was extracted from ear notch by the high salt method according to Jiang et al. (1997) and dissolved in TE solution at -20°C.

PCR amplification

Primers were designed according to the nucleotide sequence of the rabbit tyrosinase gene : P₁5'-GAGTACAATAGCCGACAGAG-3'; P₂5'-GAAATTGGC-AGCTTTGTCCAG-3'. PCR amplifications were carried out on Hybaid Express according to the following conditions: 2.5 µl of 10×reaction buffer (10 mM Tris, pH8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂), 200 µM of each dNTP, 25 pmol each primer, 0.5 unit Taq polymerase and 100 ng rabbit genomic DNA in a final volume of 25 µl. Cycling included an initial denaturation step at 95°C for 2.5 min, followed by 30 cycles 40s at 95°C, 40s at 60°C, 1 min at 72°C and a final step of 7 min at 72°C.

SSCP detection

The 881^A 881^G mutation in the rabbit tyrosinase gene can be detected by PCR-SSCP approach. In brief ,sixteen percent non-denaturing polyacrylamide (acrylamide: bisacrylamide=29:1) gel was prepared according to

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Sambrook et al. (1989), running conditions and silver staining were performed according to Jiang et al. (2000). Genotypes were determined by the numbers and the patterns of the electrophoresis bands. Genotypes of the two homozygotes displayed two single strand bands, but the positions were different. Heterozygote displayed the above four single strand bands.

Sequencing and alignment

The amplification products of the two homozygous individuals were recovered and ligated to pMD18-T vector for sequencing. The sequences were aligned with DNASIS2.5 software to examine the mutation base pair.

RESULTS

PCR amplification

The amplification product should be 174 bp in size calculated from rabbit tyrosinase cDNA. The electrophoresis result (Figure 1) for PCR amplification showed that a specific product of the same size was obtained, and it was suitable for SSCP analysis.

SSCP detection method

The 881^A 881^G mutation in the rabbit tyrosinase gene can be detected by the PCR-SSCP approach. Three genotypes of GG, GA and AA at nt 881 of rabbit cDNA, corresponding to three phenotypes of homozygous "black ear", heterozygous "black ear" and white coat color respectively could be clearly distinguished as shown in Figure 2. In this pedigree, two parental individuals (1 and 2 in Figure 2, GA) were heterozygous "black ear" rabbits and their progeny had all of the three expected genotypes (GG, GA and AA), showing obvious Mendelian segregation. The same results were obtained by analysis of other six pedigrees. Typing of 61 unrelated animals revealed that 40 GA and 8 GG rabbits had the "black ear" coat color, while 13 AA rabbits were white in coat color. Therefore, complete association between genotypes and coat color patterns was observed.

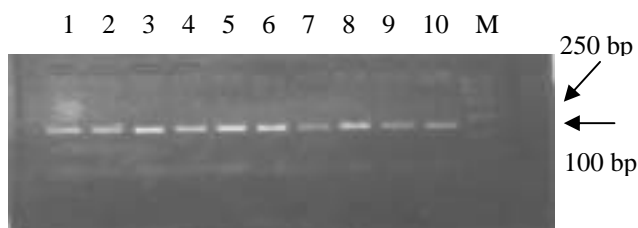


Figure 1. Amplified fragment of rabbit tyrosinase gene by PCR, Lanes 1-10, samples M is DL2000 marker.

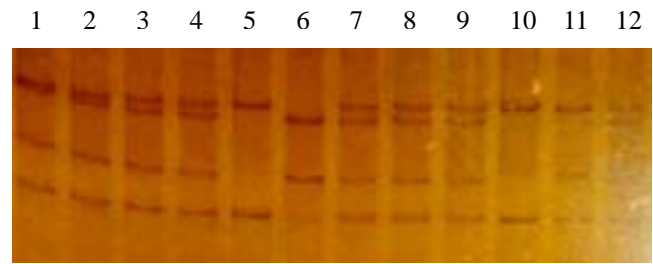


Figure 2. PCR-SSCP detection of 881^A 881^G mutation of the tyrosinase gene in a "black ear" rabbit pedigree. Genotypes of lanes 1-4, 7-9 and 12 were GA, lanes 5, 10 and 11 were GG and lane 6 was AA. 1 and 2 were parental "black ear" rabbits and 3-12 were their offsprings.

Sequencing and alignment

The PCR products of two individuals with GG and AA genotypes were sequenced and aligned. The A G mutation at nt 881 of the tyrosinase gene was confirmed from the results of sequencing (Figure 3) and alignment (Figure 4), indicating that in the "black ear" rabbits the same mechanism for coat color existed as in the California rabbits.

DISCUSSION

For many kinds of wool rabbit strains, for example Germany strain and France strain, the coat colors are exclusively white ("albino" type) and they are often difficult to distinguish from one another by wool rabbit producers. Wool rabbits with special appearances as strain-specific markers are necessary for both rabbit breeders and producers. For this purpose we have developed a high wool producing rabbit strain with "black ear" as a marker and have established a PCR-SSCP method for the determination of homozygous and heterozygous "black ear" rabbits using only one gel. As no test cross is ever needed, this method saves time and reduces cost and most important of all, accurate in determining the genotype of each rabbit.

Several mutations have been identified in the coding sequence of the rabbit tyrosinase gene and some are strain-specific (Aigner et al., 2000). The A G transition at nt 881

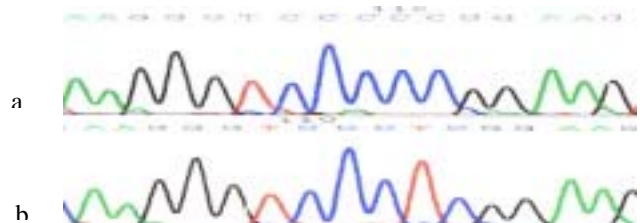


Figure 3. Sequences of tyrosinase gene involving the A G mutation in a, homozygous "black ear" rabbit and b, homozygous white rabbit.

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1   GAGTACAATAGCCGACAGAGCTTATGTAATGCAACTTCCGGGGGACCCTTGCTGCGCAAT
      |||
1   GAGTACAATAGCCGACAGAGCTTATGTAATGCAACTTCCGAGGGACCCTTGCTGCGCAAT

61  CCTGGAACCATGACAAAGCCAGGACTCCGAGGCTCCCCTCCTCATCAGATGTGGAATTT
      |||
61  CCTGGAACCATGACAAAGCCAGGACTCCGAGGCTCCCCTCCTCATCAGATGTGGAATTT

      121  TGCCTAAGTCTGACCCAGTATGAATCTGGTTCATGGACAAAGCTGCCAATTT
          |||
      121  TGCCTAAGTCTGACCCAGTATGAATCTGGTTCATGGACAAAGCTGCCAATTT

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Figure 4. Alignment of sequences of tyrosinase gene between homozygotes of “black ear” and white rabbits (upper, “black ear” rabbit; lower, white rabbit).

has been found both in Chinchilla which shows complete chinchilla , and in the California strain, the coat color of which is characteristic of “eight black” at the extremes. Another mutation of nt 1073 C T is also present in Chinchilla. After five-years’ breeding, a “black ear” wool rabbit strain was developed by intercrossing wool rabbit with *Yipulu* strain and selecting for high wool production, heavy body weight and high litter size. A distinct pigmentation pattern could be seen at the two ears and the nose, while no obvious pigmentation was observed at the other usually five black sites, namely the terminals of feet and the tail. We speculate that the same mechanism existed in the “black ear” strain as in the “eight black” California and *Yipulu* strains and, the differences lie in the temperature increase in the new strain. Therefore, a pair of primers were designed to amplify the region where the A G mutation is located to make clear whether the same mutation occurred .

PCR-SSCP is one of the most easy and practical approaches for SNP detection. Detections of the A G mutation were carried out by running the 16% polyacrylamide gel (5% glycerol included) at a constant voltage of 10 V/cm for 12 h at 4°C. Three genotypes, GG, GA and AA could be clearly distinguished. Analysis on a pedigree produced by GA×GA crossing showed that the F₁ progeny followed the Mendelian segregation pattern.

Typing of 61 unrelated rabbits showed completely association consistent between the three genotypes of GG, GA, and AA and the two phenotypes of “black ear” and white coat colors. Therefore, the A G mutation at 881 nt of the tyrosinase gene in rabbits is the cause of the “eight black” coat color of the *Yipulu* strain as well as the “black ear” coat color in the newly developed wool rabbit strain.

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