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A Rapid and Cost Effective Method for Genotyping Two Types of GHR Mutations in Sex-Linked Dwarf Chicken

[1,2]Yan Wang, [1,2]Hao Qu, [1,2]Cheng-Long Luo, [1,2]Jie Wang and [1,2]Ding-Ming Shu
[1]Guangdong Academy of Agricultural Sciences, Institute of Animal Science,
[2]State Key Laboratory of Livestock and Poultry Breeding, Dafeng 1st Street One,
Wushan, Tianhe District, 510640 Guangzhou, Guangdong, People's Republic of China

Abstract: Large deletion or mutation at the exon 10 and 3' untranslated region of Growth Factor Receptor (*GHR*) gene results in dwarf chicken and the common method for detecting mutation is direct PCR amplification. But since, the target fragment is large, the amplification is inefficient and unreliable and this process consumes a lot of expensive specific DNA polymerases. Researchers focused on the point mutation C146280A in the deletion fragment and set up a new method to detect large deletion and point mutation in this region. Comparing with direct PCR this new method which can detect both large deletion and point mutation with regular low-cost DNA polymerases is more stable, reliably and cheapness.

Key words: GHR, sex-linked dwarf chicken, genotyping, mutation, stable

INTRODUCTION

Sex-linked dwarf chicken has a smaller body size and its weight is only 60-70% of normal chicken, saving 20% feed compared with that for normal chicken. Thus, sex-linked dwarf chicken is best for hybrid production because of lower cost and higher economic benefit. It has been reported that the mutation in Growth Factor Receptor (*GHR*) gene located in the short arm of Z chromosome results in dwarf chicken phenotype which is sex-linked recessive inheritance (Burnside *et al*., 1991; Agarwal *et al*., 1994; Tanaka *et al*., 1996).

Mutations in chicken *GHR* gene affect the phenotypes (Tahara *et al*., 2009). It has been reported that large deletion of exon 10 and 3' UTR (Untranslated Region), G679T and T335C mutation in coding region, two T to C mutations with 352 bp from coding region (352+2) result in the dwarf phenotype (Agarwal *et al*., 1994; Huang *et al*., 1993; Duriez *et al*., 1993; Hull *et al*., 1999).

The large deletion of exon 10 and 3' UTR leads to a loss of 27 highly conserved amino acids in GHR extracellular domain, resulting in dwarf chicken phenotype (Agarwal *et al*., 1994). RFLP analysis revealed that 6.0 kb band from exon 10 and 3' UTR region of wild-type chicken was replaced by 4.3 kb band in dwarf chicken (Agarwal *et al*., 1994) and the common method for detecting mutation is direct PCR amplification (Halabian *et al*., 2008; Qiong *et al*., 2011). But since, the target fragment is large, the amplification is inefficient and unreliable and this process consumes a lot of expensive specific DNA polymerases.

Researchers studied the large deletion of exon 10 and 3' UTR (Type I mutant) in *GHR* gene to look for an easy, low-cost and efficient molecular detecting method. A pair of primers that are relatively easy to amplify were designed within Type I mutation and in the meanwhile they also worked for detection of C146280A point mutation in 3' UTR (Type II mutant) (Ouyang *et al*., 2008). Type I mutant of *GHR* gene is the deletion of coding region from 1744-3526 bp with a total loss of 1781 bp which is located in exon 10 and 3' UTR. Type II mutant is at 1131 bp of type I deletion fragment with C to A mutation which results in Hae III-RELP polymorphism.

MATERIALS AND METHODS

DNA preparation: In this study, all chickens came from the Guangdong Academy of Agricultural Sciences. Genomic DNA was isolated from blood samples of all chickens using a standard phenol:phenol/chloroform purification-based protocol.

Primers: The primers were designed using two end sequences of the deletion fragment in *GHR* gene Type I mutant as templates P1-F/R (P1-F:5'-GGCAGAAA-

Corresponding Author: Ding-Ming Shu, Animal Breeding and Genetics Group, Guangdong Academy of Agricultural Sciences, Institute of Animal Science, Dafeng 1st Street One, Wushan, Tianhe District, 510640 Guangzhou, Guangdong, China

GGCAGAAACCCCAAGTATGGAAATGCCTGTCCCAGACTACACTTCTATTCATATTGTTCACTCTCCACAAGGC
CTTGTGCTCAATGCAACTGCACTGCCTGTGCCAGAGAAAGAATTTAACATGTCTTGTGGCTATGTGAGCACAGA
CCAGCTGAACAAAATCATGCCG**TAG**CAATTCTTTGACTAGATGTTTGCAGTATTTCACAGCAGAGTCAGCTTGG
GCTTGTGTGCTGAAACCAAAATGAAGTCTAAACGTTTGTCGTGGTTCTTACAAGTTTATCTCGATTGTTGAAAC
ATAAAATATTCATCAAAATATTCCTCTTGTTCTGTAAATATTATCTATGAATTTGTCTTGAGACTGCTTAACTAGCT
GTGACTGTCTTGTTCTTGTGGGTGTTGATTTTTGTGATACTAAACATTGAAATGACTATGTTTAATGTACAGTAA
ATCATGCTTTTTGATAAAGCTATAGATAAGGTGGTCTAAAATCTGAGAACTTAGTAAAAATCATGCAGTTGACGG
AGATCTTTCTATCAGTAATTGGGAACTGAGCATGAAAGTGAAAAAAAAAACACAAAAATAGTTTGAATAAAAT
ATCTATTTTGATCTATATAAATTAATAAAATATATTTAATATTTTAGTCACAAAAGCTTAGATGTTTTATATTACAC
TACATGATGTAGTTTAATTATGATGGCCCATCCAAGGAATGTAAGCACTGCAGTCTTGAAAAGGAGTTTTCTGTT
TTATAAGAGTTTTGTTTGCAGCAAAAATTAAAAACAGATTTTACAGAGCCATTTTATACCTCCAAAGAAATCTGT
CGAGAAATTTTGAAATATTTTAGGAACTTAGAGTAATCG**ATCCTGTTGCTTTCTTATGG**TAACTAATATAGAAA
TAGACATGAAATTTAAAGTTTTGTAGAAAACTTTAAACTATACAAAATATTTGGACAAAAATTTCTGTTCTTCAT
CAAATTCTGGTTAATGATAATATACTTTTTTAAAGCAAGGTTTCAATGACCACAGTATTAAGTAGTATGGAAGTA
CACTTTCATTGAAAATTTTTGAAAACACATGAATAAGCTCTGGTGATTTAGCAGCATAATCTTTTCGTATTTGAC
CAACTTCTGAAGCCCTCCAATGGACAAGCTACCACTACAGTTATTAGACAGTGACACATGAAATATTGATTAAG
AAAATAAGGACTTCAGGATTTGG**C(A)** CAGCAGTTGATTTTTTAAGCTACAAATAGATTTAAAGGCTTCAACG
AAACACAGGAATTGAATACGGTGTTGTTAAAAAGGATTTTTCCTCTTTGCCTAACAGCATCTCCTCTGACTGAG
CTAGGCAGACTTCTTAGGAAAGAAAGGAGGTGAGAAAACCAAAACCAAGTGGGAAAGTTCTCTTTATTCTTA
AGTATATAAAGAACTTGACAGAGCTTTGCAATGAGTTTTTATTGCTTCAGAAGTTACCTTATTAGGCTATAGCTG
CATCATATCAGGGTTCTTCTGATAAAGCAAATGGGGATGTTCATTGCCTTTCTCTGACGTGAACAGAAGTGCAT
GACGTGCAGTGAATGCAGATCTTGCTCATGATGTCTCCCACTGAACTCACTGATGCTCTCTTTGTGAC**CAAGGG**
CTACAGGACTGAGCCCAAACATTGGGTGGGATTTTTACGTTCAGAACGTACTCTATGGTAAATGTGCATTAGG
TATTCTGCTAATTTGCTGCTATATTTTTCTAACAGCTACATTATTTAGTTACATATAAAATTTCATAGATGATAGACA
CTAATGCAAGTAAAGCCTATGTTTGTTTGGAAAAAGTTTCTTACTCTGTTTCTATTGCCAAAAATAGTTAAATAC
CTCAATCAAACTCAGAATGTCATTTTGGTACTTTACTGGTCACACAAGCCATTATTCGCCGGTCAGAAGATGTG
TCTTTCAGTTTCTATTTAACTTTCCTTATGTCAGTGTTTTGTTTGTTTTCCTCGAAGTTTAATAAATGT**ATTGTCT**
TTGATCTGTCCCGA

Fig. 1: The expected sequence of amplification product of P1-F/R and P2-F/R. The framed sequence is the stop code of chicken *GHR* gene. The sequences underlined are P1-F/R primers. The sequences double-underlined are P2-F/R primers. The shadowed sequence is the sequence lost in Type I mutant. The nucleotide with brackets is point mutation in Type II mutant (C146280A)

CCCCAAGTAT G-3', P1-R: 5'-TCGGGACAGAT-CAAA GACAAT-3'). The two end sequences of Type II mutant (reference to AC187590 of GeneBank) were used as templates for design of primers P2-F/R (P2-F: 5'-ATCCTGT TGCTTTCTTATGG-3',P2-R: 5'-CTCAGTCCTGTAGCCC TTG-3'). The sequence of expected amplification product was shown in supplemental Fig. 1.

The forward primers of P1-F/R and P2-F/R (P1-F and P2-F, respectively) were diluted to 10 μM and mixed with equal volumes of upstream primers P3-F. P1-R and P2-R were diluted to 10 μM and mixed with equal volumes of upstream primers P3-R. The P3-F/R primers were used as a new pair of primers for later experiment.

PCR amplification: The total volume of PCR reaction was 25 μL with DNA template 1 μL, 2×PCR Reaction Mix 12.5 μL, each of 10 μM P3-F/R forward and reverse primers 1 μL, Taq DNA polymerases (Dongsheng, Guangzhou, China) 0.625 U, ddH_2O 8.25 μL. The PCR conditions were preheating at 94°C for 3 min followed by 33 cycles of 94°C for 30 sec, 56°C for 30 sec and 72°C elongation for 45 sec with additional elongation at 72°C for 10 min. The products were kept at 4°C. The PCR were subjected to 1.5% agarose gel electrophoresis.

RESULTS AND DISCUSSION

The results of PCR amplification using the P3 primers are shown in Fig. 2. Three genotypes of Type I mutant

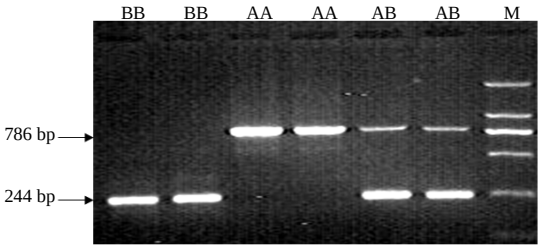

Fig. 2: The PCR amplification product with P3 primers by electrophoresis (1.5% agarose gel). AA, AB and BB represent the three genotypes of Type I mutant. M is the DNA marker (DL 2000)

were AA (a band of 786 bp) stands for homozygous wild-type alleles, BB (a band of 244 bp) represents for homozygous dwarf alleles and AB (two bands of 786 and 244 bp) stands for the heterozygous wild-type and dwarf alleles.

Currently, the detection of the mutation in *GHR* gene was achieved by direct PCR Method. Since, the target band is >2 kb (Burnside *et al*., 1991; Tahara *et al*., 2009), the amplification efficiency is low and unstable in addition to consumption of expensive specific DNA polymerases. So, the test cost is high while the efficiency is low. The results of PCR amplification using the P3 primers showed that the band of 786 bp can reply the large deletion band (1781 bp) because the 786 bp band located in the deletion band. So, researchers using the P3

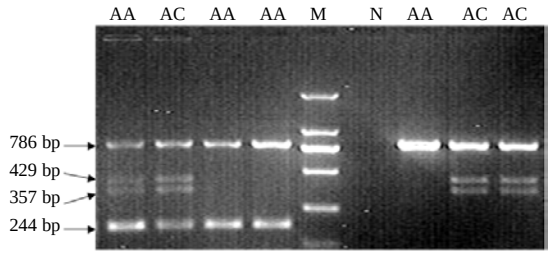

Fig. 3: The endonuclease Hae III digestion of PCR products from amplification with P3 primers (1.5% agarose). AC and AA stand for two genotypes of Type II mutant. M is the DNA marker (DL 2000)

primers to identify the large deletion mutation not only convenient and easier but also does not need expensive LA amplification polymerase.

P3-F/R primers for detecting Type I mutant can be used for detecting Type II mutant too. The single nucleotide mutation C146280A Type II mutant can be recognized by endonuclease Hae III. Further analyses of this mutation were carried out using the Restriction Fragment Length Polymorphism (RFLP) Method. Type II mutant was determined by two alleles. C is the allele with the endonuclease recognition site and A is the wild-type allele the band of 244 bp is the product of Type I mutant (Fig. 3).

Comparing with direct PCR this new method which can detect both large deletion and point mutation with regular low-cost DNA polymerases is more stable, reliably and cheapness.

CONCLUSION

In this study, the method researchers presented can detect the large deletion and C146280A point mutation in *GHR* gene. It is more convenient, easier, more stable and doesn't need expensive LA amplification polymerase which in turn lowers the test cost and improves the efficiency.

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