

Investigation of candidated genes for molecular characterization of DongGyeong dog populations (Gyeongju)

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Abstract

This study was carried out to investigate the characters of short-tailed dogs (DongGyeong dogs) with anatomical insights, molecular genetics in Gyeongju. The present study was conducted to further characterize of short-tailed dog population in Gyeongju. The short-tailed dog was analyzed in the distribution of 55 individual. The anatomical insights were by x-ray. For discovery of specific genes expressions were measured by Hot-start PCR analysis. Anatomy survey, the number of vertebral typical consists of more than 20. 88.9% of short-tailed dog populations consists of 3-8 vertebrates. The 54 individuals of the 47 observe the vestigial tailed of the sacrum. No detected sacrococcygeal vertebrae degradation individuals were malformation defects. The 3 genes were DEGs (differentially expressed genes) in Dong-Gyeong dogs. We succeeded in finding 3 novel DongGyeong dogs specific genes by using Hot-start PCR analysis, this study suggests that these novel genes may play role (s) in DongGyeong dogs.

Key words : DongGyeong, Coccygeal vertebrae, DEGs, *Ankykorbin*, *Sirt3*, *Hdac6*

INTRODUCTION

Short-tailed dogs distribute to China, South Korea and Japan. Most living environment and local culture was caused by a failure to not adapt, and the establishment of the country's only a short tail, Shilla era, DongGyeong guess that was to remain until recently (Cho et al, 2006; Ha and Kim, 1998). DongGyeong dogs was Samguksagi (12th century), trapping of DongGyeong (17th century) and a record in the excavated ruins of the 5~6th century Shilla, and it appears that South Korea's oldest indigenous animal shaped earthenware is a dog (Choi et al, 2008) (Fig. 1).

Study of native dog species were well organized by Japanese scholars after the Japanese annexation of

Korea. Jin-do dog has been designated as a natural monument since 1957. It has the best characteristics of the country's ethnic history and worth preserving despite poor management specification as endangered, and the production of hybrid pedigree because it is not being fixed (Natural heritage administration of Korea, 2006).

The oldest record in the country was DongGyeong dogs in the history. To have a family line fixing and organized population on the DongGyeong dogs to secure fixed and, if possible, designated as natural monuments. Therefore, DongGyeong dogs stock is fixed, an object can be secured, and investigate the natural breeding environment to specify the monument would be to secure the necessary materials.

Recently, the elderly population increases, due to increasing divorce, family pets are living in. deep, and pet population is continually increasing. Meanwhile, the 21st cen-

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Fig. 1. Photograph of pottery sherds with dog with Shilla (2-3th century).

Table 1. Distributions of animals used in this experiments

	Adult	Young*	Unclassified**	Total
Male	20	4	3	27
Female	22	3	2	27
Total	42	7	5	54

*Young-Less than 1 year old

**Unclassified-Not knowing the information

Table 2. Lists of primer sequence for candidate genes

Gene name	Primer sequence
<i>Ankycorbin</i>	For CCTGAGCCTTGTCGATTCTC
	Rev AGCTGCCGGATTATGACATC
<i>HDac6</i>	For CTTGCTGGTGGCCGTATTAT
	Rev GCTCCAAAGTGCTGAGAACC
<i>Sirt3</i>	For CTGGGGTGGACACAAGAACT
	Rev CCTGCACTACACCTTCAGCA

ture, an international attention has focused on biological diversity, and holds our country's unique biological resources for research and tends to be concentrated. In particular, a country around the world in his own unique ethnic characteristics for preservation of native species dog has a competitive research is underway (Cho and Cho, 2006; Halverson and Edward, 2000).

Therefore, the current South Korea at the center of Gyeongju as the breeding DongGyeong dogs were approximately 60. Gyeongju City Hall is supported in part by the economic and breeding farms, but in DongGyeong dogs do not have a system of records and research. In such



Fig. 2. Photographs of the DongGyeong dogs appearance, hair color is a varied as the white, yellow, black, brindle.

a background in social DongGyeong dogs for the preservation of biological resources DongGyeonggi try to secure the basic database for anatomical and molecular genetic characteristics of biological research.

MATERIALS AND METHODS

Animals population

The populations of short-tailed dogs, DongGyeong dogs guess that the Gyeongju-city hall been given financial support to 22 breeding farms in the clinical state of health is good, more than four months old to 54 (Table 1). DongGyeong dogs characteristics common to coccygeal vertebrae and the number of radiation through biological tests, coccygeal vertebrae an array of forms, sarcococcygeal vertebrae and anatomical attributes the varied as a colored with white, yellow, black, brindle dogs (Fig. 2).

Observations and anatomical factors for investigation

The general identifiable information (gender, age, weight, body length, body height), the number of radiation checks through the coccyx, the appearance of the tail, sacro-

coccygeal vertebrae vestigial tailed of existence, sacro-coccygeal vertebrae fusion of the existence of the investigation, respectively. For DongGyeong dogs common external morphology, which is suspected as a unique form of a short tail or the tail DongGyeong dogs with a x-ray films capture device to examine the diagnostic radiology equipment for examine sacrococcygeal vertebrae.

Selection of random primer sequence for PCR

The Primer 3 CGI ver 0.2 program for the future and the creation of a specific gene expression pattern of mouse and human genomic research on the application of research to sequence the previously reported for nucleic acid binding, transcription regulation, mRNA processing, chromatin related factor associated with GC contents 60% or more of the gene to be produced (Table 2). Meanwhile, Primer size was the 20~24bp the production into the annealing temperature of 60°C to ensure their production. And through the program to determine a primer NCBI database to check back into the sequence, Bioneer Inc to assess the production.

DNA extraction

DongGyeong dogs and a variety of species to the specific gene expression pattern was observed for blood collection and 5ml. Included in 5ml a collection of blood anticoagulant EDTA (ethylenediamine tetra-acetic acid) to be treated as the genomic DNA extraction (Ha et al., 1998). DNA extraction methods were DNA purification kit (Nucleogen Biotechnology, Seoul, Korea; Cat No. 1512) to purchase and used. This approach is in the blood supply kit for 200µl proteinase K will be using the protein degradation and lysate buffer from 10 minutes to eliminate the handling of 56°C. Finally, DNA extract was used 99% ethanol. DNA extracted from a kit so that supply is binding through the spin column. And both times after washing the remaining steps to remove impurities

and refining. To extract DNA present in the final column for elution buffer (10mM Tris Cl; pH 8.5) got to use. The extracted DNA is used to keep until -20°C.

Hot start-polymerase chain reaction (PCR)

The extracted DNA using the polymerase chain reaction 10mM Tris-HCl (pH 9.0), 1.5mM MgCl₂, 30mM KCl, stabilizer and tracking dye, 250uM dNTP (dATP, dCTP, dGTP, dTTP), and that includes 1U Taq DNA polymerase with *AccuPower* PCR premix (Bioneer Inc; Daejeon, Korea). PCR conditions are non-specific sequence of binding to prevent hot start-PCR method used his five minutes at 94°C predenaturation 94°C, who responded in one minute after denaturation, 60°C in 45 seconds annealing, 72°C from 40 to 45 seconds extension. Postelongation repeat reaction times had conducted 72°C in 10 minutes. This research master gradient PCR machines for use in Eppendorf resignation.

Electrophoresis and image analysis

Amplified PCR products 20µl to a 1.5% agarose gel with EtBr (ethidiumbromide) 120V/20min electrophoresis in order to stain. The UV illuminator (Vilberlauret Inc, Germany) was observed in the band. DNA size marker is 100bp ladder (Bioneer Inc, Daejeon, Korea) is used. Amplified PCR products were the result of electrophoresis. Finger-Shotgun 7K Image Analyzer (Centronic Inc, Daejeon, Korea) is used to determine the gene expression pattern.

RESULTS

Evaluation of radiograph about the number and type of coccygeal vertebrae

The coccygeal vertebrae in the form of a radiological evaluations of the total 54 tail-shaped object were observed.

Table 3. The numbers of coccygeal vertebrae in experimental animals

No. of coccyx	0	1	2	3	4	5	6	7	8	9	10	11	12
No. of animals	1	1	1	6	6	7	12	7	10	0	2	0	1
Detective rate (%)	1.9	1.9	1.9	11.0	11.0	13.0	22.2	13.0	18.5	0	3.7	0	1.9
Cumulative rate (%)	1.9	3.8	5.7	16.7	27.7	40.7	62.9	75.9	94.4	94.4	98.1	98.1	100

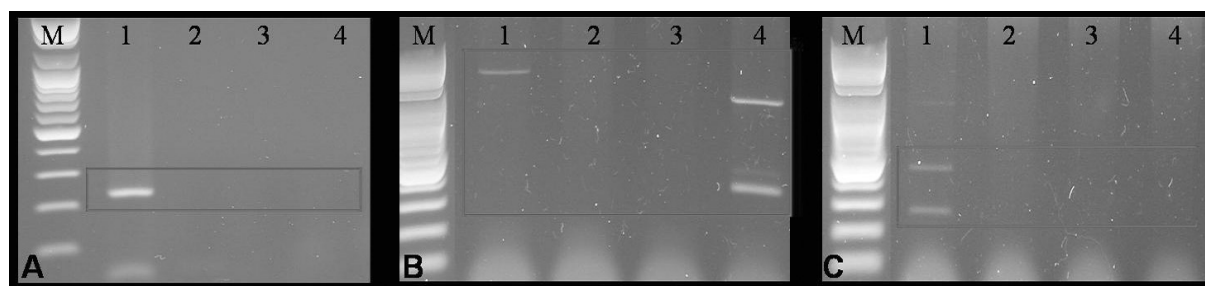


Fig. 3. Expression patterns of novel specific genes into various dogs DNA. A. *ankykorbin* B. *Sirt3*. C. *Hdacs6*, M: 1kb DNA ladder, 1: Blood DNA of *DongGyeong* dog, 2: Blood DNA of *Shepherd*, 3: Blood DNA of *Huschi*, 4: Blood DNA of *human*.

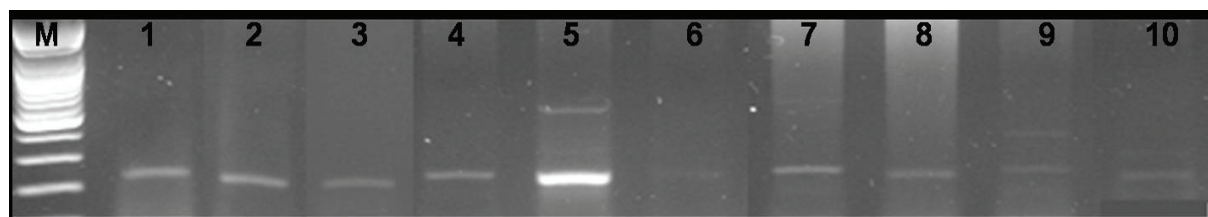


Fig. 4. Expression of *ankykorbin* gene in short tailed dog populations and other species dogs. M: 1kb DNA marker 1-10: individual species.

Curl tail is 12, lined tail is 42 percent. 27 males in a curl tail eight lined tail is 19, 27 females, the curl tail was investigated for four and 23 are lined tail, total 54 the number of coccyx, on average, 5.9 (0~12) If the 6-coccygeal vertebrae 12 individuals (22.2%), eight coccyx of 10 individuals (18.5%), 3~8 in the coccygeal vertebrae 54 of the 48 individual cases are investigated as 88.7% in the first place (Table 3).

Discovery of specific novel gene in DongGyeongji

The DongGyeong dogs, shepherd, huschi, human blood using DNA extracted from 22 species mentioned gene expression using primer know the difference between the saw. Each object in the above experiment through the genomic DNA pool drew homology with a primer on using a mouse to perform PCR, each object is carrying DNA from a specific primer on the DNA binding and only the specific expression band pattern of finding a molecular genetics and genetic information and the discovery of the band were diverse pattern observed by the advent of the genome pool to determine the differences can take advantage of this approach would be to use.

After the identification of the genetic information of indigenous human and other mammals homologs of the gene analysis can be utilized to feed the modified RAPD (randomly amplified polymorphic DNA) to be performed. 22 kinds of primer, using a total of three differentially expression is a kind of gene that seems to have been observed (Fig. 3).

The DongGyeong dogs, the only unusual gene expression (*ankykorbin*) in a collective expression used to determine an object. The gene for the future will need to do more research, DongGyeong dogs object to the extraordinary manifestations that would be used as marker. The *ankykorbin* a total of 30 of the 10 gene expression were detected (Fig. 4).

DISCUSSION

Typically, the spine of anatomical structure, the skeleton of the last components of coccyx, on average, 6-23 field (Dyce et al, 2002; Sisson et al, 1975). The *shepherd* with a long lined tail, the appearance of the typical array of coccygeal vertebrae seemed to look at the number of

coccygeal vertebrae. *Welchi-Corgi* was normally showed short tail with coccygeal vertebrae bone and the *Schipperke* has several coccygeal bones as a short tailed dog (Bennett and Perini, 2003). DongGyeong dogs can see an array of suspected radiation biology of anatomical DongGyeong dogs fixed pedigree checks are as basic material for research is very significant.

Radiation was based on photographs taken of the results of short-tailed dogs coccygeal vertebrae result of a number of observations. Most of the 88.7% the number coccygeal vertebrae 3~8. If the human eye to people's non-tailed got coccygeal vertebrae radiation shooting average of 4~5 at the coccyx, the average number of experimental dogs when compared to 5.9 because there is a near non-tailed shape blunted tail is supposed. The vestigial tailed coccygeal vertebrates was not showed 7 individuals. There is an appeared with malformation and physiological defects.

In anatomical approach, the common aspects of coccygeal vertebrae consists of more than about 20, but the group estimated that 88.9% surveyed in short tailed dogs populations (DongGyeong dogs) consist of 3~8 coccygeal vertebrates. Also, 54 of the 47 to observe the vestigial tailed of the coccygeal vertebrae and non-vestigial tailed animals were detected malformation defects. The DongGyeong dogs is not a form of biological malformations. Registered dogs from FCI (Federation Cynologique Internationale) were non-tailed and docking tail, Species will be able to admit one object.

In molecular approach of candidate genes, the first candidate gene *ankykorbin* was named retinoic acid 14, and this gene is well known in genetics, the object of other species (Peng et al, 2000), which are places for more people to use genetic information obtained from the genome database, chimpanzees, mice, rat, avian, between the fish homology to verify. Compared to the human and chimps, dogs showed only 90 percent or more of the high homology. In fact, several species of these genes are preserved in the ability to perform reliably. The *ankykorbin* is highly concentrated at cortical actin cytoskeleton structures in terminal web and cell-cell adhesion sites and stress fibers. The *ankykorbin* appears to be an actin cytoskeleton associated protein

and may be involved in actin cytoskeleton maintenance and/or reorganization.

The second candidate gene *Hdac 6* (histone deacetylase 6) was chromatin remodeling related gene known other species (Seigneurin-Berny et al, 2001; Zhang et al, 2003), and the object of genetic as well conserved in the genome of NCBI database to determine whether to take advantage of the country. By comparing the human homolog and the dog appears to 82.9%. In fact, these dogs are currently unknown, and the very highly homolog large animals (chimpanzees, pigs, cows), called on different candidates genome.

In eukaryotes, the DNA in the cell nucleus is bound by histones and other chromosomal proteins to form a highly organized and compact structure called chromatin. A large body of evidence has demonstrated that chromatin plays a critical role in regulating gene expression. A number of post-translational protein modifications such as acetylation, methylation, ubiquitylation or phosphorylation of histones and other nuclear proteins create a code that orchestrates the organization and function of chromatin (Jenuwein and Allis, 2001).

The third candidate gene *sirt 3* (silent information regulator 2) was apoptosis and cell growth related gene in other species (Jin et al, 2009), and the genetic as well conserved in the genome of NCBI database to determine. By comparing the human homolog and the dog appears to 79.6%. The silent information regulator 2 (Sir2 or sirtuin) proteins comprise a family of NAD⁺-dependent protein deacetylases and ADP-ribosyltransferases that are highly conserved in both prokaryotes and eukaryotes (Duntnall and Phillus, 2001). Sirtuins are involved in many cellular processes including gene silencing, 2 cell cycle regulation, metabolism, apoptosis (Luo et al, 2001). Mammals express seven sirtuins (North and Verdin, 2004), SIRT 1, 2, 3, and 5 possess deacetylase activity, whereas SIRT 4 and 6 are ADP-ribosyltransferases. These proteins display a range of subcellular localization, including nuclear (SIRT 1, 6, 7), cytosolic (SIRT 2)(North et al, 2003), and mitochondrial stores (SIRT 3, 4, 5) (Michishita et al, 2005). SIRT3 is a key mitochondrial protein deacetylase proposed to play key roles in regulating mitochondrial metabolism (Jin et

al, 2009).

The external features of DongGyeong dogs was one of the tail of the skeleton bases vestigial tailed or regression related genes involved in apoptosis and cell growth. the comparative analysis of the survey was in line for homologs. After analyzed DNA, extracted from blood of DongGyeong dogs and other dogs, Discovery with candidate differentially expressed genes. Further the genes will be functional researches. Also, DongGyeong dogs populations and other species (total 30) were the analyzed using the candidate gene. As a result of various genes expressions to observed.

In conclusion, securing historical significance, formatting to secure evidence of regional or local breeding, genetic traits and appearance more than five uniqueness of conservation, establishment of standard varieties (shapes, characteristics, specifications, seeking, temperament etc.), population size, isolated breeding facilities, management specification program, records management, in more than 10 varieties of its three-year-old group formed, the research results can be achieved if you have natural conditions as a monument to be desperately trying to do.

The DongGyeong dogs identified anatomically normal vestigial tailed of the skeletal structure. In molecular genetics, we found differentially expressed genes in DongGyeong dogs with coccygeal vertebrae. therefore, we should proceed to the study of genes. also, we need to secure individuals fixed pedigree.

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