Medical Xpress

The genetic cause of a severe skeletal disease in Brazilian Terrier puppies revealed

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The genetics research group led by Professor Hannes Lohi, based at the University of Helsinki and the Folkhälsan Research Center, has, in collaboration with Adjunct Professor Kirsi Sainio's research group, discovered the cause of a life-threatening skeletal disorder affecting Brazilian Terriers. The disease is caused by a mutation in the GUSB gene. Malfunction of the GUSB gene has previously been linked to a severe skeletal disorder in humans, called type VII mucopolysaccharidosis (MPS VII).

The gene discovery is yet another example of a shared disease heritage between dogs and humans. Based on this study a gene test has been developed for the breed to eliminate the disease.

The study has been published in *PLoS ONE* journal on July 5, 2012.

Bone abnormalities are common in a group of lysosomal storage diseases, also known as mucopolysaccharidosis (MPS). Lysosomes are microscopic intracellular organelles that consist of more than 40 different degradative enzymes. They function as a cellular "cleaning system" and are responsible for example digesting foreign bacteria in the cells and destroying damaged cellular components. Functional defects in the lysosomal enzymes lead to the accumulation of a particular structural component, glycoaminoglycan, in the lysosomes, and this in turn leads to the development of skeletal disorders known as mucopolysaccharidoses.

Eleven different MPS diseases have already been found in humans.

Typical features of the disease include dwarfism, skeletal abnormalities, coarse facial features, cloudy corneas and overgrowth of the internal organs.

Dogs are known for extreme structural variation, and some of these features have been intentionally bred. For example, the length of the limbs varies greatly between breeds. Besides breed-specific particularities, dogs also have serious inherited skeletal disorders, the genetic backgrounds of which are now being discovered.

The diagnosis of the skeletal disorder in Brazilian Terriers confirmed by gene discovery

A few years ago our research group was introduced to Brazilian Terrier puppies that had severe congenital skeletal abnormalities in the limbs, loose joints, facial deformities and dwarfism. The affected puppies could not move and had to be put down before the second month of life.

Subsequent appearance of several affected litters led to a thorough characterization of the clinical, pathological and genetic features of this unknown disease. The disease is caused by the same gene (GUSB) that causes type VII mucopolysaccharidosis in humans (MPS VII or Sly syndrome) and the symptoms of the affected Brazilian Terriers resemble closely the human disease.

"The Brazilian Terrier is a rare breed and is mainly found in its country of origin, Brazil, and in Finland. This recessive disease has been an unfortunate problem in the small breed since the affected puppies are really never even able to get on their feet. We conducted various clinical and radiological tests in the Animal Hospital of University of Helsinki, as well as tissue analyses, which indicated that the skeletal growth of the affected puppies is delayed and the ossification centers may even be

completely missing in the normal growth areas. Since many bone diseases are similar to each other, the final diagnosis of this disease was confirmed only after the gene discovery," explains Developmental Biologist, Adjunct professor Kirsi Sainio.

Mutation was identified by a next-generation sequencing method

A genome-wide study in pedigree dogs was conducted to identify the genetic cause of the disease. The comparison of the genomes of 7 affected and 11 unaffected dogs mapped the disease gene into an extensive region of the chromosome 6, containing more than 220 genes.

"Mapping the disease gene to a specific chromosomal region was in itself a breakthrough for the project, but finding the actual mutation among 200+ genes with conventional methods would have been a laborious and time-consuming process. This is the first time that we have successfully applied a novel next-generation sequencing approach to identify the causative mutation. Instead of analyzing the region gene by gene, we were able to capture and read the entire region simultaneously in the selected samples. The mutation was quickly discovered and subsequently confirmed in over 200 dogs," says the leader of the study, Professor Hannes Lohi.

The MPS VII disease in Brazilian Terriers is caused by a mutation that causes changes an amino acid, P289L, in a beta-glucuronidase enzyme. This causes a structural abnormality and inactivates the enzyme. The beta-glucoronidase enzyme is responsible for the lysosomal degradation of key components of the extracellular matrix in the bones, and if not functioning, causes the accumulation of these components in the lysosomes. Components of extracellular matrix are also secreted into urine, as was discovered in the affected dogs.

Gene test helps breeding programs

MPS VII in Brazilian Terriers is a single-gene recessive disorder. According to the study, as many as every third dog carries the mutation. However, the study enabled the development of a DNA test that allows to identify carriers and keep them in the breeding programs. Carrier dogs are not affected by the disease. It is recommended that breeder not breed two carrier dogs, but instead combine them with dogs that are free of the mutation.

"With the help of the gene test, this disease can now be systematically eliminated from the breed, without having to exclude carrier dogs from the breeding programs. This is important especially in small dog breeds to maintain genetic diversity. Active breeders have made this study possible by participating in the study with their dogs. In turn they now benefit from the new gene test," says MSc Marjo Hytönen. The gene test is available from Genoscoper (www.genoscoper.com).

More than 450 genetic diseases that include different types of developmental skeletal disorders have been found in humans. Bone diseases are highly heterogeneous and classified based on clinical, radiological, biochemical and genetic findings. Hundreds of genes linked to human skeletal diseases have already been found. The core building block of the bones is an extracellular matrix, which consists mainly of proteoglycans and collagens. Therefore, most mutations have also been found in genes affecting the formation of extracellular matrix in skeletal structures. Genetic findings are being used to describe different groups of skeletal diseases such as collagenopathys and osteogenesis imperfecta.

Provided by University of Helsinki