The search for canine obesity-related genes and the effects of genetic mutation on metabolism in dogs

Summary of Doctoral Thesis

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Introduction

Obesity is a condition that the energy intake exceed energy expenditure, and excessive fat is accumulated to white adipose tissues. Two kinds of factors are related to obesity, such as environmental and genetic factors. Environmental factors includes daily lifestyle such as dietary habits and exercise quantity. Genetic factors depend on genetic polymorphism which influences the metabolism. As obesity-related genes in human beings, uncoupling proteins (UCPs), leptin, leptin receptors, beta-2 and beta-3 adrenergic receptors (ADRB3) genes have been reported. Recent years, G protein-coupling receptor 120 (GPR120) has been added to the list.

GPR120 is a fatty acid receptor relating to hormonal secretory functions such as glucagon-like peptide-1 and cholecystokinin. Gene polymorphisms are reported in human GPR120, especially the His270Arg mutant is functionally related to obesity. GPR120 is expressed in the lung, jejunum, ileum, colon, hypothalamus, hippocampus, spinal cord, bone marrow, skin and white adipose tissue in rodents and humans.

ADRB3 is a subtype of adrenergic receptors, which is expressed mainly in adipocytes. When adipocytes were stimulated with adrenergic agonists, acyl-glycerol would be broken down into glycerol and fatty acids, then causes heat production. In humans with Trp64Arg mutated ADRB3, the resting metabolism decrease by 200-220 kcal compared to wildtype people, and that increases risks of obesity and diabetes.

In veterinary medicine, obesity is the most common nutritional disorder as in human medicine. Epidemiological studies have revealed that one third or fourth of dogs are overweight or obese in developed countries. Furthermore, Obesity is a risk factor of pancreatitis, hyperlipidemia and arthritis in dogs. However, there is no report investigating obesity-related genes in dogs. In the present study, we focused on two candidate genes, such as GPR120 and ADRB3, and analyzed the relationship between their genetic polymorphism and body condition scores in dogs.

We have searched for SNPs of GPR120 (chapter 1) and ADRB3 (chapter 2) in client-owned dogs, and investigated the relationship between their gene frequency and body condition scores. In Chapter 3, we have developed a cell expression system which expresses ADRB3 mutants, and compared their molecular functions.

Chapter 1: Analyses of canine GPR120, a possible obesity-related gene in dogs.

GPR120 is a member of the free fatty acid receptor family, which assumes long chain and unsaturated fatty acids as ligands. Fatty acids are not only energy source as substrates of beta-oxidation, but also signaling molecules in various cellular functions. It is reported that GPR120-deficient mice have developed obesity with fatty liver and insulin intolerance following a high fat diet feeding. In human GPR120 studies, higher gene frequency of the Arg270His mutant is detected in obese people, and the signaling function is attenuated, so this mutant is thought to be a risk factor of obesity.

In this chapter, we have cloned canine GPR120 cDNA and revealed the molecular nature. We have explored single nucleotide polymorphisms (SNPs) of GPR120 in 141 patient dogs' genome DNA, and investigated the relationship with obesity. Focus is on finding the candidate obesity-related genetic variations of cGPR120.

Cloned canine GPR120 consisted of 1,086 bases including ORF. Furthermore, canine GPR120 was 84-95 % identical to those of the human, mouse, rat, cat, horse, pig, and white bear. They were comprised of 361 amino acids, and the homology of the amino acid sequences were 78-96%. The highest identity was found to cats, and the lowest to rats. Tissue distribution analysis revealed that canine GPR120 was expressed in the lung, jejunum, ileum, large intestine, hypothalamus, ippocampus, spinal cord, bone marrow, skin, and adipose tissue.

We have analyzed GPR120 genomic sequences of 141 dogs, and found 5 synonymous and 4 non-synonymous SNPs. Gene frequencies of c.287T>G (Leu96Arg) variant was 0.125 in all dogs (n=141) and 0.500 in beagle dogs (n=36). The variant c.595C>A (Pro199Thr) was detected in 40 of 141 dogs tested, and the gene frequency was significantly higher in overweight and obese dogs than that in normal dogs ($p=0.022$).

The purpose of this chapter was to discover the genetic variants of canine GPR120 as candidate obesity-related genes. We have cloned canine GPR120 cDNA, and revealed the tissue distribution according to rodents and human studies. We have found 4 non-synonymous SNPs, especially the gene frequency of c.595C>A (Pro199Thr) was significantly higher in overweight and obese dogs suggestive of that the variant is a candidate obesity-related gene in dogs.

Chapter 2: Analyses of canine ADRB3, a possible obesity-related gene in dogs.

Beta 3-adrenergic receptor (ADRB3) is a subtype of adrenergic receptors, which has a seven times transmembrane structure and G protein-coupling structure expressed mainly in the white adipose tissue. When adipocytes were stimulated with adrenergic agonists, acyl-glycerol would be broken down into glycerol and fatty acids, then causes heat production. In human beings, when the 189th base of the ADRB3 gene mutates from thymine to cytosine, the 64th amino acid constituting ADRB3 mutates from tryptophan to arginine. In this mutant, the resting metabolism decrease by 200-220 kcal compared to wildtype people, and that increases risks of obesity and diabetes.

In this chapter, we have explored single nucleotide polymorphisms (SNPs) of ADRB3 in 160 patient dogs' genome DNA, and investigated the relationship with obesity. Focus is on finding the candidate obesity-related genetic variations of ADRB3.

We have analyzed ADRB3 genomic sequences of 160 dogs, and found 5 synonymous and 7 non-synonymous SNPs. Gene frequency of c.749C>T (Ser150Phe) was 0.194, detected in 13 dog breeds including Yorkshire terrier and Miniature dachshund which are reported easy to grow fat, and it was significantly higher in overweight and obese dogs than that in normal dogs ($p=0.0001$). Gene frequency of c.1121C>G (Pro374Arg) was 0.053, detected in 7 dog breeds including Yorkshire terrier and Miniature dachshund, and it was significantly higher in underweight dogs than that in normal dogs ($p=0.0001$). Gene frequency of c.1121C>G (Pro374Arg) and c.1184A>C (Pro395Gln) were 0.053 and 0.697, detected in 7 and 17 dog breeds, respectively.

The purpose of this chapter was to discover the genetic variants of canine cADRB3 as candidate obesity-related genes. We hve found 7 non-synonymous SNPs, especially the gene frequency of c.749C>T (Ser150Phe) was significantly higher in overweight and obese dogs, and that of c.1121C>G (Pro374Arg) was significantly higher in underweight dogs, respectively. It was suggested that these variants are candidate obesity-related genes in dogs.

Chapter 3: Development of a cell expression system of canine ADRB3 variants, and their functional analyses.

In chapter 2 and 3, we have discovered candidate obesity-related variants in canine GPR120 and ADRB3 genes, but the data depend on epidemiological research and functions of the receptors have not ever been demonstrated. In human ADRB3, *in vitro* studies using a cell expression system of Trp64Arg variants have been performed and it was revealed that this mutation induces attenuated intracellular cyclic AMP production. Moreover, metabolic rates in humans with this mutation decreased by 200 kcal compared to those with wild type (WT) variants based on *in vivo* studies. Also in the present study, it is the next step to analyze functions of these mutants if they cause changes in metabolism

In this chapter, we have developed a cell expression system of ADRB3 variants (WT, Ser150Phe, Pro374Arg, Pro395Gln and empty) with HA-tags and demonstrated functional analyses by reference to human studies. Western blotting analyses using anti HA tag antibody detected ADRB3 proteins in 4 mutants (WT, Ser150Phe, Pro374Arg, Pro395Gln), but not in the mock one.

Subsequently, we have stimulated these cells with adrenergic agonists (adrenaline,

noradrenaline, CL316,234, IBMX and RO20-1724) for 30 minutes and measured intracellular cAMP concentrations. Adrenaline, noradrenaline and CL316,234 induced cAMP production in the cells expressing ADRB3, but not in the mock mutants. Amounts of produced cAMP were tended to be lower in the Ser150Phe and Pro395Gln mutants compared to WT, but not in the Pro374Arg mutant.

The purpose of this chapter was to develop a cell expression system of ADRB3 variants, and analyze the difference in their functions. The Ser150Phe and Pro395Gln variants showed attenuated intracellular cAMP production when they were stimulated with adrenergic agonists, suggestive of that these mutations may decrease metabolic rates and increase the risk of obesity in dogs.

Conclusion

We have analyzed GPR120 and ADRB3 as candidate obesity-related genes in dogs. Gene frequency of c.595C>A (Pro199Thr) variant of GPR120 was significantly higher in overweight and obese dogs than that in normal dogs. Gene frequency of c.749C>T (Ser150Phe) variant of ADRB3 was significantly higher in overweight and obese dogs, and that of c.1121C>G (Pro374Arg) was significantly higher in underweight dogs than that in normal dogs. Cell expression studies of ADRB3 variants have revealed that Ser150Phe and Pro395Gln mutants caused decrease in adrenergic agonists-induced intracellular cAMP production. These variants may be obesity-related mutations. Considering these variants as gene test items and utilizing the results for nutritional management, it, may be helpful for treatment and prevention of obesity in dogs