Study of DNA Polymorphisms in Canine Uncoupling Protein 2 and 3 genes

Abstract of Doctoral Thesis

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Uncoupling proteins (UCPs) are members of a family of anion-carrier proteins that localize to the mitochondrial inner membrane and play an important role in energy homeostasis. Genetic association studies indicate that human *UCP2* and *UCP3* variants are associated with obesity, insulin resistance, type 2 diabetes mellitus, and metabolic syndrome. However, the biological functions of these genes in dogs are not as well documented as are those in humans. Therefore, we identified DNA variants of canine *UCP2* and *UCP3* and examined potential association between these variants and metabolic data.

We isolated dog *UCP2* and *UCP3* cDNAs from skeletal muscle; each cDNA contained a partial 5'-untranslated region and an entire open reading frame. Canine genomic and transcript sequences indicated that canine *UCP2* comprised exons 1 to 8 and canine *UCP3* comprised exons 1 to 7. The nucleotide and predicted amino acid sequences and the genomic structures of human *UCP2* and *UCP3* were highly homologous to the canine orthologs. We identified not only a full-length *UCP3* cDNA but also a *UCP3* cDNA that lacked exon 3 in dogs. The mRNA expression patterns of *UCP2* and *UCP3* were determined based on analysis of total RNA extracted from each of 30 canine tissues. Canine *UCP2* and *UCP3* mRNA expression profiles were similar to those of humans. Therefore, canine UCP2 and UCP3 may play a role in the regulation of energy metabolism, as do the human orthologs.

Using genomic DNA samples, we directly sequenced the coding regions and a portion of the intronic regions of *UCP2* and *UCP3*, including exon-intron boundaries, from 119 different dogs that represented 11 breeds. We identified 10 SNPs (nine within introns and one within a 5'untranslated region) and four indels (all within introns) in *UCP2* sequences and 13 SNPs (11 within introns, one missense, and one silent) and one indel (within a 3'untranslated region) in *UCP3* sequences.

We further examined potential associations between *UCP2* and *UCP3* variants and metabolic data (GLU, T-Cho, LDH, TG) and dog breed. In an analysis of 50 Labrador Retrievers; none of the *UCP2* variants was significantly associated with GLU, LDH, TG, or T-Cho levels; in contrast, four *UCP3* polymorphic sites in intron 1 were significantly associated with T-Cho levels. Based on an interbreed analysis involving 30 Shibas and 30 Shetland Sheepdogs, the frequencies of alleles at five sites (four SNPs and one indel) within *UCP2* differed

significantly between these two breeds, as did alleles at four SNPs within *UCP3*.

The results obtained from a limited number of individuals indicated that *UCP3* in dogs may be associated with total cholesterol levels. Examination of larger sample sizes and other analyses will lead to increased precision of these results.