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Analysis of Na, K-ATPase  $\beta$ 1 isoform gene of canine erythrocytes associated with hereditary high Na, K-ATPase activity

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Normal canine erythrocytes contain low potassium (K) and high sodium (Na) concentrations and lack Na, K-ATPase activity (LK). However, some Japanese dogs genetically have erythrocytes characterized with a high activity of Na, K-ATPase, which results in high K and low Na concentrations in their erythrocytes (HK). It has been determined that dog reticulocytes had considerable amounts of Na, K-ATPase, but lost it rapidly during maturation into erythrocytes. In contrast, reticulocytes from HK dogs showed similar regression of Na, K-ATPase during maturation, but they retained a high activity of enzyme even after maturation. In the present study, the conformational and quantitative differences of Na, K-ATPase  $\beta$ 1 isoform gene between HK and LK dogs were examined, and constitution of  $\beta$  subunit in these dogs was estimated. The results were as followed;

1. A mutant  $\beta$ 1 isoform gene was detected in HK dogs. The sequence of the mutant gene showed a single nucleotide substitution from G to A at the position 614 which causes an amino acid

change from Ser<sup>205</sup> to Asn. Moreover the normal  $\beta$ 1 isoform gene was also detected in HK dogs. These results indicated that the  $\beta$ 1 isoform gene of HK dog was presented as a heterozygous form.

2. Northern blotting hybridization analysis using specific probe for  $\beta$ 1 isoform gene showed that the  $\beta$ 1 isoform mRNA in HK reticulocytes was more abundant than that in LK reticulocytes. However it could not be confirmed whether the difference caused by the mutant gene or not.

3. There was no difference in the isoform of Na, K-ATPase  $\beta$  subunit between HK and LK reticulocytes. The  $\beta$  subunit in canine reticulocytes was clarified as  $\beta$ 1 isoform.

These results suggested that the mutant  $\beta$ 1 gene in HK dogs might synthesize an abnormal Na, K-ATPase which is not affected by the regression mechanism of the enzyme during erythroid maturation in LK dogs. This might be possible explanation for the high activity of Na, K-ATPase in HK erythrocytes.