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Analysis of R-type pyruvate kinase gene of canine erythrocytes
associated with hereditary high Na, K-ATPase activity

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Normal canine erythrocytes completely lack Na, K-ATPase activity, resulting in intracellular low potassium (K) and high sodium (Na) concentrations (LK). In contrast, there are certain Japanese and Korean breed dogs that have erythrocytes characterized by inherited high Na, K-ATPase activity, with high K and low Na concentrations (HK). It has been reported that the activity of erythrocyte pyruvate kinase (PK) is significantly higher in HK dogs than in LK dogs. In the erythrocytes of HK dogs, also abnormal PK isoenzyme has been recognized besides R-type PK which is dominantly expressed in mature erythrocytes. In the present study, the canine R-type PK gene was analyzed to clarify whether the abnormal PK activity in the erythrocytes of HK dog depends on the gene defect or not. The results were as followed.

1. Three amino acid changes associated with four nucleotide substitutions were detected in R-type PK gene of HK dogs, compared with the previously reported PK gene in Basenji dogs. However, these substitutions were also found in LK dogs.
2. Since the previously reported PK gene in

Basenji dog was not determined whole amino acid coding region in R-type PK, the undetermined region was sequenced and whole amino acid sequence of canine R-type PK was confirmed in the present study. The amino acid sequence of canine R-type PK was highly homologous with that of human or rat. However, there was no difference between the amino acid sequences of R-type PK in HK and LK dogs.

3. The nucleotide sequence of the first intron in canine R-type PK gene was determined. While the nucleotide sequences of this region between HK and LK dogs were found to be the same, highly homologous sequences with the promoter regions and the first exon of L-type PK in rat.

These results indicated that abnormal PK activity in the erythrocytes of HK dogs was not due to the gene defect of R-type PK. Since there have been some enzyme activity disorders in erythrocytes of HK dogs, the abnormality of PK activity in HK dogs might depend on the extraordinary mechanism for the generation of erythrocytes.