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Analysis of the Na,K-ATPase 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 cells). However, some Japanese dogs genetically have erythrocytes characterized by high activity of Na,K-ATPase, which results in high K and low Na concentrations in their erythrocytes (HK cells). It has been determined that dog reticulocytes initially have considerable amounts of Na,K-ATPase, but lose it rapidly during maturation into erythrocytes. In contrast, reticulocytes from dogs having HK cells show similar regression of Na,K-ATPase during maturation, but they retain high activity of the enzyme even after maturation. In the present study, the conformational difference of Na,K-ATPase between HK and LK cells was examined, and polymorphism of genomic DNA was compared. The results were as follows;

1. The α -subunit gene of canine erythrocyte Na,K-ATPase was amplified by reverse trans-

cription polymerase chain reaction (PCR). As a template, RNA extracted from dog erythroblasts was used. Nucleotide and deduced amino acid sequences of the α -subunit gene of HK cells were determined using PCR products and compared with those of LK cells. Four nucleotide mutations were identified, while the amino acid sequences of the α -subunit of HK and LK cells were found to be the same.

2. The polymorphism of canine genomic DNA was analyzed using a specific probe for the α -subunit gene. In this analysis, no obvious difference between HK and LK cells could be found.

These results indicated that the presence of Na,K-ATPase in HK cells was not due to amino acid substitution in the α -subunit of the Na,K-ATPase. Further analysis is necessary to clarify the cause of the Na,K-ATPase remaining in HK cells.