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# Korean variant dogs have red blood cells with high amino acid accumulation

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**Abstract** : Amino acids, Na, K and glutathione (GSH) in red blood cells (RBCs) and hematological indices were examined in Korean dogs. A total of seven dogs possessing RBCs with high K and high glutathione (GSH)(HK/HG) were found in 42 Korean dogs : three from Cheju dog, two from Jindo dog and two from Korean mongrel. The RBCs in Korean HK/HG dog contained abnormally high aspartate (Asp), Glu and glutamine (Gln) the same as in HK/HG RBCs from Japanese Shiba dog. Two dogs possessing RBCs with HK and low GSH (HK/LG) were found in Cheju dog, and they accumulated Asp and Gln. Thus, not only the existence of HK dog was confirmed in Korean dogs, but HK/LG dog was also found. The Asp concentration in RBCs from seven of 33 LK dogs was more than 1000μmol/lc, the same as in variant LK RBCs with defective Glu/Asp transport (LK/GAT), while it was less than 800μmol/lc in normal LK RBCs. Thus, there were variant dogs having RBCs with abnormally high amino acids accumulation among HK and LK Korean dogs.

**Key words** : aspartate, dog, high K, glutamate, red blood cells.

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## Introduction

Dog and other carnivore red blood cells (RBCs) differ from other animal RBCs in their cellular cation composition: the composition is low K and high Na (LK) due to the lack of Na, K-pump in carnivore, whereas it is high K and low Na (HK) in other animal[1~5], Dog RBCs are also unique in having Na-dependent glutamate (Glu)/aspartate (Asp) transport[6,7]. The Na-dependent Glu/Asp transport was enhanced in inherited variant dog RBCs with HK and high glutathione (GSH)(HK/HG), because of a steep inward Na gradient[8,9]. In HK/HG RBCs, there were abnormally high Glu, Asp and glutamine (Gln), concentration, which were attributed to Glu/Asp influx[8,9]. GSH concentration was also increased because of the cellular high Glu concentration which overcame the feedback regulation of GSH synthesis[10].

However, dogs having RBCs with HK but low GSH (HK/LG) were found among Japanese Shiba dogs, and their Glu/Asp transport activity was low or defective[11,12]. In HK/LG RBCs, though the concentration of Asp and Gln was increased, Glu was not accumulated and GSH synthesis was regulated. After a defect in Glu/Asp transport was detected in HK/LG RBCs, the defect was also found in RBCs from several LK dogs[13]. There were at least two kinds of variant RBCs; one with an abnormality of residual Na, K-pump, namely HK, and the other with defective Glu/Asp transport [14]. These variants were found independently or simultaneously in the same dog, and the inherited mode of both variants was recessive[13].

As mentioned above, the amino acid accumulation accounted for their enhanced Glu/Asp influxes in HK/LG RBCs[8,9]. Therefore, these amino acids were not considered to accumulate in the RBCs with defective Glu/Asp transport, However, in the RBCs with the defective Glu/Asp transport, amino acids were accumulated; Asp and Gln were high in HK/LG RBCs, and Asp was high in LK RBCs with defective Glu/Asp transport (LK/GAT). Though the defect had no clinical manifestations, it may be the result of some metabolic disorder of amino acids. The defect was found in the Japanese Shiba dog, and some were found in the HK variant. HK gene penetrated many Japanese dog breeds and populations, and also several Korean dogs[15]. Thus, there was the possibility that there might be variant RBCs with defective Glu/Asp transport in Korean dogs.

To clarify the clinical meaning and/or physiological polymorphism, not only the mechanism for the defect but its geographical extent must be examined. It would be very interesting to examine whether there are dogs having variant RBCs with abnormal amino acid accumulation in Korean dogs. Here we examined the amino acids, Na, K and GSH in RBCs and other hematological indices in Korean dogs.

## Materials and Methods

Dogs from two breeding colonies in Cheju Island, Korea, and a group kept by Cheju University were used: total 42 dogs from 10 Jindo dogs (3 males, 7 females), 13 Cheju dogs (5 males, 8 females) and 19 Korean mongrel dogs (10 males, 9 females). Blood was drawn by venipuncture into tubes containing heparin.

Hematological indices such as RBC count, hemoglobin (Hb) and MCV were measured by an automated counter, and PCV was measured by the microcapillary method. Na and K were measured by flame photometer as reported earlier[16]. In brief, 50Él of blood was added to tubes containing 110mM $MgCl_2$ buffered with 10mM Tris-HCl layered on a bed of n-butyl phthalate. Then the tube was centrifuged and the pellets were hemolyzed in a solution containing detergent (Triton X-100), $NH_4OH$ and $CaCl_2$[17]. The cations in the solution were measured by flame photometer (Shimadzu-AA-6701/6601) at wave length 589nm for Na and 766nm for K. GSH was measured using 5,5'-dithiobis(2-nitrobenzoic acid) according to the method of Beutler[18]. The concentration of amino acids in RBSc was determined by HPLC as described elsewhere[19]. Washed cells were first deproteinated with 10% $HClO_4$ and neutralized with 2N KOH. The solution was centrifugated, the supernatant collected, and the supernatant was then mixed with the fluorescent reagent, o-phthalaldehyde. The mixed solution was applied to HPLC: Japan spectroscopic (JASCO) Model 801-SC HPLC with a JASCO 820-FP spectrofluorometer and re-

versed-phase column (C-18, 4.6mm×250nm).

## Results

Concentrations of Na, K and GSH in RBCs and hematological indices from Korean dogs are indicated in Table 1.

Table 1. Concentrations of Na, K and glutathione (GSH) in red blood cells and several hematological indices in LK, HK/HG and HK/LG dog in Korea

| Items | LK* | HK/HG* | HK/LG** |
|---|---|---|---|
| Na (mmol/lc) | 113±7 | 8.1±0.8 | 7.6±0.2 |
| K (mmol/lc) | 8.32±1.14 | 106±13 | 103±11 |
| GSH (mmol/lc) | 2.33±0.46 | 9.58±2.63 | 4.10±0.69 |
| RBC (/$10^6$) | 5.77±0.85 | 5.23±1.15 | 6.02±0.21 |
| Hb (g/dl) | 12.3±2.0 | 10.6±2.7 | 11.9±0.4 |
| PCV (%) | 41.9±5.7 | 36.2±9.4 | 37.8±0.8 |
| MCV (fl) | 64.7±2.9 | 69.2±2.5 | 64.0±0.3 |

* means and SD in LK (n = 19 for Na, K and GSH, n = 9 for hematological indices) and HK/HG (n = 7 for Na, K and GSH, n = 4 for hematological indices) dog RBCs.
** means and ranges from two dogs in HK/LG dog RBCs.

Dogs were classified into three groups according to their cation composition and GSH concentration in RBCs; LK, HK/HG and HK/LG RBCs. In this report, the RBCs in which the GSH concentration was more than two-fold the highest value in LK dog was referred to as HG (GSH concentration 〉 5.8mmol/lc), and RBCs fewer than that were called LG (GSH concentration 〈 5.8mmol/lc). The mean GSH value in HK/HG RBCs was 4.1-fold higher than that in LK RBCs, while that in HK/LG RBCs was 1.8-fold higher than in LK RBCs. Thus, the GSH value in HK/LG was higher than in LK RBCs, but the mean was less than half that in HK/HG RBCs. The indices of RBCs such as cell count, Hb and PCV indicated that these values in HK/HG dogs were slightly lower than those in LK and HK/LG dogs, though there were no significant differences. MCV in HK/HG dogs was larger by 7 and 9% compared to LK and HG/LG RBCs, respectively.

As indicated in Table 2, a total of 7HK/HG dogs (3 males, 4 females) were found among 42 Korean dogs: three from Cheju dog (1 male, 2 females), two from Jindo dog (2 females) and two from Korean mongrel (2 males). Moreover, two dogs with HK/LG RBCs were found in Cheju dog (2 males). Thus, not only was the existence of HK dog confirmed, but HK/LG dog was also found among Korean dogs. The Glu, Asp and Gln levels were determined in Korean dog RBCs as shown in Fig 1. The ranges of these amino acids in each dog group reported elsewhere were also indicated in the figure. In the HK RBCs, the mean concentrations of cellular Asp, Glu and Gln were around 10-fold higher than in normal LK RBCs. However, the range of Glu concentration was wider than in previous reports; the Glu concentration in one of 7HK/HG dogs was less than 2,000μmol/lc (arrowtipped line in Fig 1B). However, the lowest Glu concentration in the HK/HG RBCs was 941 μmol/c, which was twice that in the highest LK RBCs (Fig 1A). The GSH concentration in the HK/HG dog RBCs was slightly low compared to the other HK/HG RBCs with Glu concentration more than 2,000μmol/lc. Actually, the GSH concentration in the dog RBCs was 6.75mmol/lc.

Table 2. Incidence of LK, HK/HG and HK/LG in Korean dog groups.

| Dog group | Cheju | Jindo | Korean mongrel |
|---|---|---|---|
| LK | 8 | 8 | 17 |
| HK/HG* | 3 | 2 | 2 |
| HK/LG* | 2 | 0 | 0 |
| Total | 13 | 10 | 19 |

* HK RBCs in which GSH concentration was more than two-fold the highest value in LK RBCs were classified as HK/HG, and those with less than that were classified as HK/LG.

In HK/LG RBCs from two dogs, the GSH concentration was 4.79 and 3.41mmol/lc, respectively (Fig 1C). Glu concentration in the HK/LG RBCs did not accumulate to the level in HK/HG RBCs, but it was lower than 130μmol/lc and within the range in LK RBCs. However, the Asp and Gln levels in HK/LG RBCs were clearly higher than in normal LK RBCs (Fig 1C). These values are comparable with previous values in HK/LG RBCs (arrow-tipped vertical line

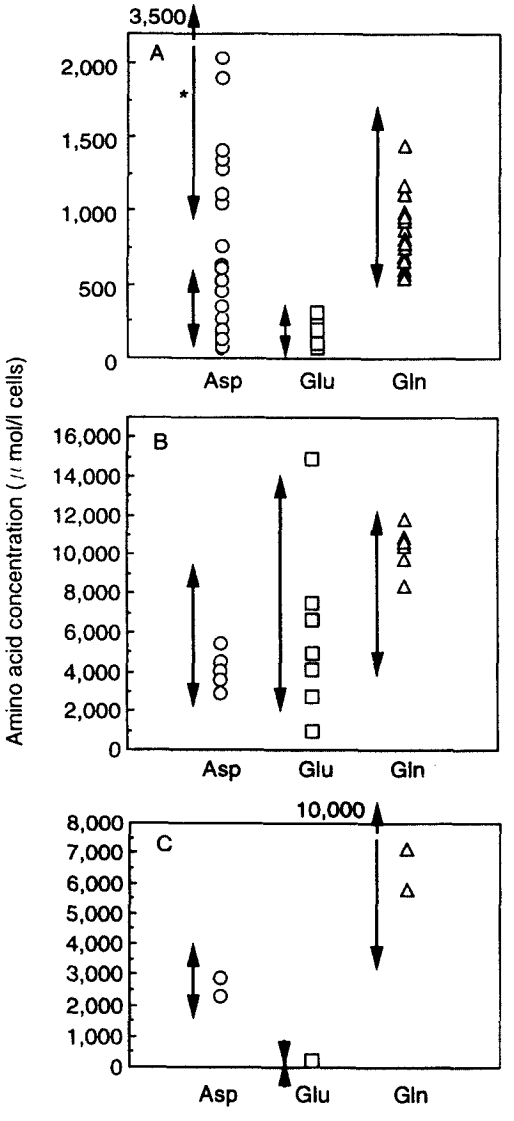


**Fig 1.** Cellular amino acid concentration for aspartate (Asp, circles). glutamate (Glu, squares) and glutamine (Gln, triangles) in LK (A), HG/HG (B) and HK/LG (C) dog red blood cells. Arrow-tipped vertical lines indicate the amino acid concentration range in each type of RBCs from dogs found in Japan and reported elsewhere (RefNo. 10, 11, 21).
* Range of Asp in LK/GAT⁻ RBSs reported (RefNo. 14).

in Fig 1C). This, dogs having HK/LG RBCs with high Asp and Gln were found in Cheju dog in Korea.

Asp concentration in RBCs from Korean LK dogs ranged widely from 50 to 2000μmol/lc. In RBCs from seven (3 males, 4 females) of 33 LK dogs, it was more than 1000 μmol/lc, comparable to LK/GAT⁻ RBCs from Japanese Shiba dog in which it ranged from 1000 to 35000μmol/lc (arrow-tipped vertical line with asterisk mark in Fig 1A). However, their Glu and Gln concentration was not highly accumulated, and the values were same as that in the RBCs from normal LK dogs. The other 26LK dog RBCs contained Asp ranging from 51 to 750μmol/lc; this concentration range was slightly larger than for normal LK RBCs, but it was almost the same (arrow-tipped vertical line in Fig 1A). However, the Glu and Gln levels in the LK RBCs with high Asp did not show any high accumilation, and the concentration range was the same as for normal LK RBCs.

## Discussion

Previously, an HK dog in Korean was reported by one of the present authors[15]. HK variant was found in several Japanese and Korean breeds or populations, whhile the variant was not found in dog groups from other countries in East Asia[15], or in western dog breeds[20]. Therefore it was deduced that the variation might arise in the dog groups at the dead end of their migration to the Far East, around Korean and Japan. Thus far, HK/LG and LK RBCs with abnormally high amino acid accumulation were reported only in Japanese Shiba dog[11,13,14]. Thus, it was revealed in this paper that variant dogs possessing RBCs with high amino acid accumulation existed in the both HK and LK dogs in Korea.

Glu/Asp transport was defective in the LK/GAT⁻ and HK/LG RBCs from Japanese Shiba dog[12,13]. Normal LK RBCs having Glu/Asp transport were named LK and Glu/Asp transport positive (LK/GAT⁺), while LK/GAT⁻ was used for the LK RBCs with defective Glu/Asp transport. The Asp concentration in LK/GAT⁻ RBCs was clearly higher than in LK/GAT⁺ RBCs in our previous report[14], and so were Korean LK RBCs with high Asp accumulation (Fig 1A). Thus, the LK dog possessing RBCs with abnormal accumulation with Asp found in Korea was probably a variant dog having RBCs with defective Glu/Asp transport; in other words, the RBCs might be LK/GAT⁻. Thus, the variant possessing

RBCs with defective Glu/Asp transport is thought to have spread to both the LK and HK Korean dogs.

In HK/HG RBCs, the enhanced amino acid influx accounted for the abnormally high amino acid accumulation[8,9]. However, the influx was defective in HK/LG and LK/GAT$^-$ RBCs, so the defective amino acid influx could not explain the high accumulation with the amino acids in the RBCs. Another explanation is needed for the contradiction between the amino acid transport and the accumulation. Though the mechanism of abnormal accumulation of these amino acids in LK/GAT$^-$ and HK/LG RBCs could not be explained well, the accumulation itself was considered due to a defect of efflux of these amino acids and some unknown defect(s) in amino acid metabolism[14].

RBCs in these variants contained several fold higher concentration of the amino acids compared to normal LK dog RBCs. The change in concentration of these substrates may affect the cellular amino acid metabolism. So it is very important to clarify the reason and role for this accumulation in the variants. As reported previously, the HK variant was only found in Japan and Korean, but not in other Asian and Western dog breeds or populations. It is also very interesting that the variant dogs having RBCs with amino acid accumulation were found in the Far East, in Japan and Koera. These variations might arise in these area.

The values for hematological indices such as RBCs, PCV and Hb were rather low in Korean HK/HG dogs alike as in the Japanese HK/HG dog[9,11,21]. Although a detailed explanation can not be given at this time, it is considered due to the hemolytic tendency in HK/HG RBCs[8,9]. MCV of Korean HK/HG RBCs was also higher than that in LK and HK/LG RBCs the same as in Japanese HK/HG RBCs[11,21]. With aging, the HK/HG RBCs lose their system for regulatory volume decrease[22], and their cell volume was larger than the other group RBCs. They were more sensitive to hemolytic toxins such as oxidant and onion[23-27]. There are probably more onion and garlic in Korean food, and Korean dogs have frequent opportunities to eat these alliums as their food. So, there may be some tactics to escape these disadvantages for RBCs in Korean dog. It is interesting that the indices in HK/LG RBCs were in the same range as LK RBCs, but not HK/HG RBCs. In future, we intend to examine these variants in terms of the correlation between environmental and inherent factors and the defect(s). In this report, we revealed the geographic extent of these variants.

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