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# Inherited Macrothrombocytopenia in a Cavalier King Charles Spaniel

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**Abstract :** A 1-year-old intact male Cavalier King Charles Spaniel was presented with a ruptured anal sac. On routine preanesthetic screening tests for surgical resection, the thrombocytopenia was observed by an impedance-type autoanalyzer. A peripheral blood smear was used as a follow-up test and giant platelets were seen on the smear. DNA assay of this patient confirmed that the cause of the platelet abnormalities in this patient was genetic mutation. To our knowledge, this is the first case report of macrothrombocytopenia confirmed based on the DNA assay results, in a Cavalier King Charles Spaniel in Korea.

***Key words*** **:** Cavalier King Charles Spaniel, dog, DNA assay, macrothrombocytopenia.

## Introduction

The Cavalier King Charles Spaniel (CKCS) is a breed with various genetic disorders such as mitral valve insufficiency, syringomyelia, episodic falling, hip dysplasia, patellar luxation, hearing loss and eye diseases. Another congenital hereditary disease of the CKCS, which has only been reported relatively recently is 'macrothrombocytopenia' (2). Macrothrombocytopenia is characterized by a decrease in the number of platelets in the peripheral blood and an increase in the size of the platelets. This disorder is associated with an autosomal recessive hereditary disease of the CKCS (3). Even if the platelet count is decreased and the platelet size is increased, hemostatic function is usually normal (11). This report is to describe the first case of macrothrombocytopenia confirmed by DNA assay in CKCS in Korea.

## Case

A 1-year-old male CKCS was presented with a ruptured anal sac. There were no remarkable findings on physical examinations. Prior to surgical resection, routine screening tests were performed. Except for a low platelet count, all hematology test results were within normal ranges (Table 1). The platelet count measured with an autoanalyzer (Celltac Alpha MEK-6450K; Nihon Kohden, Tokyo, Japan) was $2 \times 10^3/\mu L$, which is extremely low compared with the reference range (reference range, $200\sim500 \times 10^3/\mu L$). However, all other measured coagulation parameters evaluated as normal (Table 2).

To confirm whether the platelet count measured with the autoanalyzer was correct, a peripheral blood smear was prepared and a manual count performed. On the smear, the platelets appeared the same size or larger than the erythrocytes (Fig 1). A manual count yielded a platelet count approximately $150 \times 10^3/\mu l$, markedly increased compared with the automated count.

An EDTA whole-blood sample of this patient was submitted to the Department of Pathobiology at Auburn University, where a DNA assay for detection of a genetic mutation that leads to giant platelet formation is available. The result was "affected" which confirmed that the cause of the platelet abnormalities in this patient was genetic problem.

The dog showed no abnormal bleeding before or after surgery, and no mitral valve insufficiency or other possible congenital disease were observed until 13 months of age.

## Discussion

Macrothrombocytopenia in the CKCS has been associated with a genetic mutation. Previous study researching 16 CKCS families showed an autosomal recessive inheritance pattern for macrothrombocytopenia (5), and genetic studies revealed that a mutation in the gene encoding β1 tubulin is related to the macrothrombocytopenia seen in this breed. This mutation consists of a single nucleotide (G to A) change in the gene, which leads to replacement of asparagine with aspartic acid in a highly conserved area of the protein. This change likely affects microtubule stability resulting in altered platelet formation by megakaryocytes (7,8,11).

As described here, macrothrombocytopenia is characterized by a decrease in the number of platelets in the peripheral blood and an increase in the size of the platelets. The median diameter of giant platelets usually ranges from 2.5 to 3.75 μm, while that of normal platelets is 1.25 to 2.5 μm. Previous studies have reported platelets larger than 4 μm in 79% of CKCS dogs (7) as well as exceptional giant platelets larger than 6 μm (2).

Because of the increased size of macrothrombocytes, the platelet count measured with typical impedance-type auto-

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**Table 1.** Hematologic test results of this case

| | Variables | Results | Reference range |
|---|---|---|---|
| CBC | RBC ($10^6$/μL) | 6.59 | 5.5-8.5 |
| | HGB (g/dl) | 16.6 | 12-18 |
| | HCT (%) | 47.9 | 37.0-55.0 |
| | MCV (fL) | 72.7 | 60-77 |
| | MCH (pg) | 24.2 | 19.5-24.5 |
| | MCHC (g/dl) | 34.7 | 32.0-36.0 |
| | PLT ($10^3$/μL) | 2 | 200-500 |
| | WBC ($10^3$/μL) | 13.6 | 6.0-16.9 |
| | Lymphocytes ($10^3$/μL) | 4.6 | 1.0-4.8 |
| | Granulocytes ($10^3$/μL) | 8.5 | 3.0-11.8 |
| | RDW (%) | 13.6 | 12.0-16.0 |
| | MPV (fL) | 10.2 | 6.7-11.1 |
| SC | Na (mEq/L) | 146 | 141-152 |
| | K (mEq/L) | 4.6 | 3.8-5.0 |
| | Cl (mEq/L) | 117 | 102-117 |
| | ALT (μ/L) | 23 | 17-78 |
| | ALKP (μ/L) | 152 | 47-254 |
| | BUN (mg/dL) | 25.1 | 9.2-29.2 |
| | Creatinine (mg/dL) | 0.5 | 0.4-1.4 |
| | Glucose (mg/dL) | 114 | 75-128 |
| | Total protein (g/dL) | 6.1 | 5.0-7.2 |
| | Albumin (g/dL) | 3.0 | 2.6-4.0 |
| | Cholesterol (mg/dL) | 196 | 126-359 |
| | Calcium (mg/dL) | 12.1 | 9.3-12.1 |
| | Phosphorus (mg/dL) | 5.0 | 1.9-5.0 |
| | Total bilirubin (mg/dL) | 0.2 | 0.1-0.5 |
| | Amylase (μ/L) | 979 | 200-1400 |
| | Lipase (μ/L) | 142 | 10-160 |
| | AST (μ/L) | 37 | 17-44 |
| | GGT (μ/L) | 5 | 5-14 |
| | Triglyceride (mg/dL) | 46 | 30-133 |
| | Ammonia (Ug/dL) | 70 | 16-75 |
| | CRP (mg/L) | < 9 | < 9 |

CBC, complete blood count; SC, serum chemistry; ALT, alanine transaminase; ALKP, alkaline phosphatase; BUN, blood urea nitrogen; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; CRP, C-reactive protein; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; PLT, platelet; WBC, white blood cell; RDW, red cell distribution width; MPV, mean platelet volume.

mated analyzers is easily underestimated (11). With these instruments, the cell type is established according to the volume of the cell determined by the electrical impedance produced when the cell passes through the orifice, therefore giant platelets are falsely miscounted as erythrocytes (1). To avoid misevaluation, manual counting using hemocytometer should be performed.

Even if the platelet count is decreased and the platelet size is increased, hemostatic function is usually normal. Thus, this disease may be considered "incidental" or "benign" (3). As was seen in this case, the hemostatic screening tests were normal except for the platelet count and there were no bleeding episodes during or after surgery. Therefore, if a CKCS displays clinical hemorrhage, other potential causes should be investigated.

**Table 2.** Results for parameters used to assess hemostatic ability of the CKCS with low platelet count

| Variables | Results | Reference range |
|---|---|---|
| BMBT (min) | 2.5 | 2.61 ± 0.48 |
| APTT (s) | 60 | 60-93 |
| PT (s) | 12 | 11-14 |
| FDP (mmol/L) | 2.5 | 0.00-5.00 |
| D-dimer (μg/ml) | 0.2 | 0.0-0.25 |

BMBT, buccal mucosal bleeding time; APTT, activated partial thromboplastin time; PT, partial thromboplastin time; FDP, fibrin degradation product.

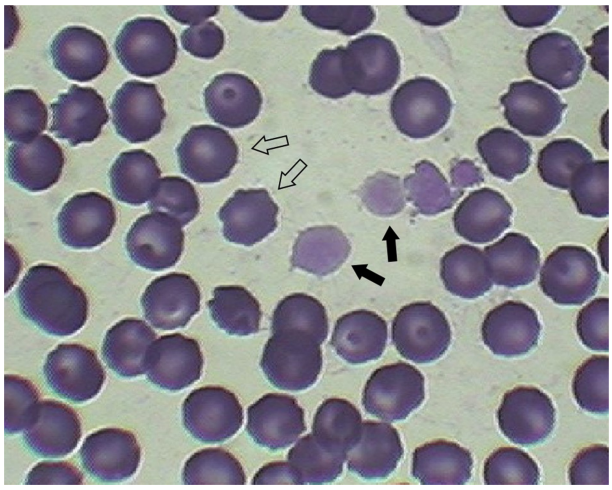

**Fig 1.** A photomicrograph of peripheral blood smear. Giant platelets (black arrows) are seen, which are approximately the same size as mature red blood cells (white arrows). (Diff-Quick, × 1,000).

In humans, primary mitral valve prolapse, which is similar to mitral valve dysplasia (MVD) of small breed dogs, is related to the activation of platelets and the short life span of platelets. Severe mitral valve regurgitation appears to be related to impaired platelet activity in dogs as well as people (6,11). To date, the clinical associations between MVD and macrothrombocytopenia in the CKCS remain unclear (8) and further investigation is needed.

Although the pathology and the correlation with other genetic conditions are not clearly defined, the prevalence of this disease has been relatively well surveyed. The prevalence of this congenital disease has been reported as approximately 30% - 50% in the CKCS population in the United States (2). In addition, another study has shown a prevalence of thrombocytopenia was about 51.43% and macrothrombocytes of 33.33% (3). However, until recently no report

could be found of this condition in a CKCS in our country.

In conclusion, macrothrombocytopenia, the disease in which the size of platelets is increased and the number is decreased is an autosomal recessive hereditary disease of the CKCS. This is the first report of inherited macrothrombocytopenia confirmed by DNA assay in a CKCS in Korea.

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