



## Diagnosis of Severe Protein C Deficiency Confirmed by Presence of Rare *PROC* Gene Mutation

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### ABSTRACT

Protein C (*PROC*) deficiency is caused by mutations in the *PROC* gene on chromosome 2q14.3. Patients with *PROC* deficiency typically present distinguished purpura, intracerebral and intravascular coagulopathy, and ophthalmologic complications. Here, we report a rare severe form of *PROC* deficiency resulting from a compound heterozygosity in *PROC*. The patient was a 5-day-old female neonate born at 39 weeks of gestation with a birth weight of 2,960 g. She was transferred to our hospital with running a fever at 38.5°C and with dark red patches on her feet. At admission, a complete blood count showed no specific findings, but levels of *PROC* and protein S were abnormally low (1% and 68%, respectively). Magnetic resonance imaging revealed intracerebral hemorrhaging and parenchymal damage with dysplasia of the brain. Ophthalmologic examination revealed vitreous hemorrhaging with retinal detachment. Genetic testing revealed a missense mutation (Arg211Trp) and a frame-shift mutation (Gly239Serfs\*8) in *PROC*, inherited from the father and mother, respectively. The patient recovered from purpura after undergoing ventriculoperitoneal shunting and treatment with fresh frozen plasma, warfarin sodium, and *PROC* concentrate. This is the first report of severe neonatal *PROC* deficiency with purpura fulminans, vitreous hemorrhage, and intracerebral hemorrhage confirmed via *PROC* genetic testing, which identified a rare compound heterozygosity of *PROC*.

**Key Words:** Protein C deficiency, Fulminans purpura, Genetic testing, *PROC* gene

### INTRODUCTION

Congenital protein C (*PROC*) deficiency is a well-documented autosomal dominant disorder of variable penetrance. Over 150 mutations have been reported<sup>1</sup>. *PROC*, located on chromosome 2q14.3 is approximately 10,802 bp and contains 9 exons (1,790 bp), 8 of which encode the protein<sup>2-4</sup>.

Severe *PROC* deficiency (*PROC* activity <1 IU/dL) is a rare autosomal recessive disorder; its chief presentations include neonatal purpura fulminans and severe disseminated

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intravascular coagulation (DIC) with concomitant venous thromboembolism<sup>5</sup>). The incidence of clinically significant *PROC* deficiency is estimated to be 1 in 20,000<sup>6</sup>). In this study, we describe the first genetically verified case of severe *PROC* deficiency in Korea.

## CASE REPORT

The patient was a 5-day-old female neonate, born by vaginal delivery at 39 weeks of gestation with a birth weight of 2,960 g. The patient is the first child of a mother with no remarkable medical nor obstetric history (G1, P1, A0, D0, L1). No unusual events were reported during the first 3 days after birth; however, on the 4th day, the patient was admitted to the hospital after her body temperature rose to 38.5°C. On the 5th day, the skin on her feet turned dark red (Figure 1). When she was transferred to our hospital, she appeared acutely ill but her vital signs were within normal range (blood pressure: 78/44 mmHg, heart rate: 136 beats per minute, respiratory rate: 55 breaths per minute, body temperature: 37.5°C).

Laboratory analysis revealed a white blood cell count of 20,410/ $\mu$ L, hemoglobin of 12.6 g/dL, hematocrit of 36%, and platelet count of 219,000/ $\mu$ L. Liver and kidney function, C-reactive protein levels, and the results for blood, urine, stool, and cerebrospinal fluid culture were normal. Cerebrospinal fluid analysis revealed a red blood cell count of 210,000/ $\mu$ L, white blood cell count of 11,730/ $\mu$ L, total protein of 2,271 mg/dL, and a glucose level of 22 mg/dL; the findings were suggestive of the presence of a hemorrhage. Blood coagulation tests showed a prothrombin time of 13.4 s (normal range: 10.0-15.3 s), activated

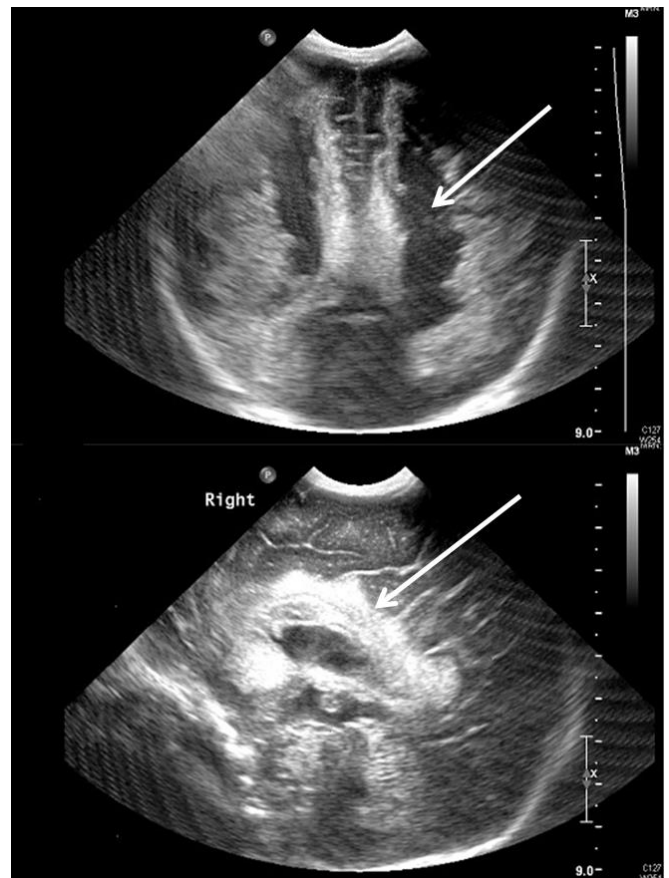


**Figure 1.** The purpura of feet at 5 days of age.

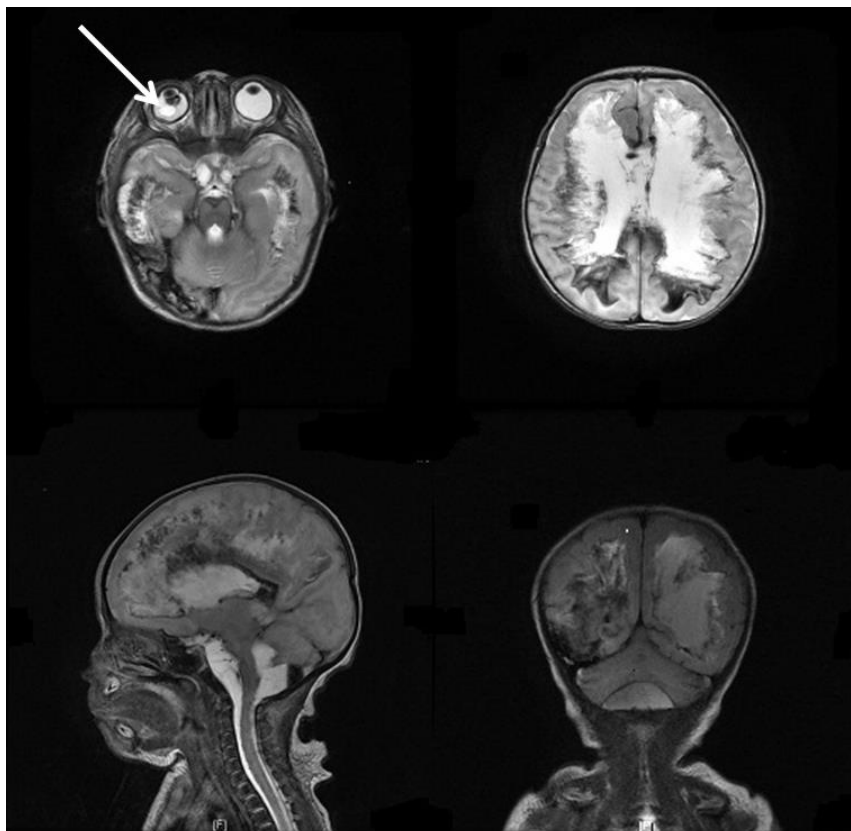
partial thromboplastin time of 44.2 s (normal range: 25.4-59.8 s), fibrinogen level of 58 mg/dL (normal range: 162-462 mg/dL), D-dimer level of 62.5  $\mu$ g/mL (normal range: 0.0-0.5  $\mu$ g/mL), *PROC* level of 1% (normal range: 50-150%), protein S level of 68% (normal range: 80-120%), and antithrombin III level of 95% (normal range: 41-95%).

Neurosonography (Figure 2) and magnetic resonance imaging (MRI) of the patient's brain (Figure 3) at 10 days of age revealed brain parenchymal destruction, intracranial hemorrhage, and atrophic change in the right eyeball. Examination of the fundus revealed posterior synechia.

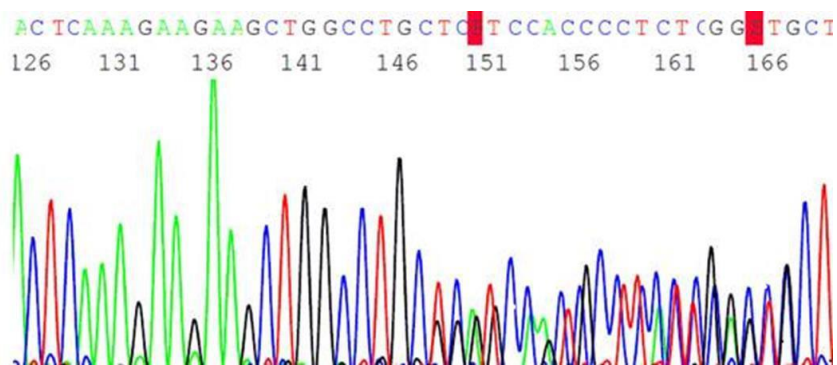
Genetic testing revealed 2 types of mutations in *PROC* (Figure 4): a missense mutation (Arg211Trp) and a frameshift mutation (Gly239Serfs\*8) causing deletion of residues 715-724. Arg211Trp has been associated with diseases and is often observed within the Korean population; however, Gly239Serfs\*8 has not been previously reported in Koreans. Genetic testing of the parents revealed that the father was a carrier for Arg211Trp and the



**Figure 2.** Neurosonogram showing symmetric hyper-echogenicity and extensive cystic parenchymal destruction communicated to ventricles (arrows).



**Figure 3.** Brain magnetic resonance imaging (MRI) shows: subacute extensive hemorrhages in the bilateral periventricular and subcortical white matter; parenchymal destruction with leukomalacic changes; communication with lateral ventricles; atrophic change of the right eyeball (arrow), with septated fluid collection and hemorrhage in the right vitreous cavity.



**Figure 4.** Genetic testing of the patient found a missense mutation (Arg211Trp) of the protein C gene, and a frameshift mutation (Gly239Serfs\*8).

mother was a carrier for Gly239Serfs\*8. Subsequently, the patient was confirmed to be compound heterozygous for *PROC*, which resulted in the severe *PROC* deficiency.

The patient received the following treatment. Fresh frozen plasma (FFP; 10 mg/kg) was administered twice a day for 28 days: at 5 days of age, from 10 days to 27 days of age, and from

32 days to 40 days of age. Warfarin sodium was administered at 100 IU/kg at 30 days of age, followed by administration of 50 IU/kg every 12 hours for the next 5 days, and finally at 0.2 mg/kg from 34 days of age. The level of *PROC* increased to 37% during the treatment period, but decreased to 10% after cessation of treatment and the patient was considered to be recovered from

the purpura.

The patient underwent extracapsular cataract extraction via phacoemulsification and synechiolysis at 3 months of age. The post-hemorrhagic hydrocephaly was also treated by external ventricular drainage and ventriculoperitoneal shunting at 5 months of age. She was discharged at 6 months of age and her *PROC* levels, prothrombin time, and activated partial thromboplastin were reassessed every 6 months.

## DISCUSSION

The incidence of asymptomatic and clinically significant *PROC* deficiency has been reported to be 1 in 200-500 and 1 in 20,000 healthy individuals, respectively<sup>5,7</sup>. Although there have been several cases of purpura fulminans in the Korean population that were suspected to be due to *PROC* deficiency, none were genetically confirmed via identification of mutations in *PROC*<sup>8</sup>.

Diagnostic testing for *PROC* deficiency typically relies on functional assays such as chromogenic assays containing snake venom, clotting assays, and enzyme-linked immunosorbent assays<sup>5</sup>. However, in addition to these assays, genetic testing for mutations in *PROC* located on chromosome 2q14.3 was also needed<sup>9</sup>.

For a healthy term infant, the average *PROC* level is 40%, with mild deficiency diagnosed for values up to 20%, moderate-to-severe deficiency at 1-20%, and severe deficiency at <1% or if *PROC* is undetectable<sup>5,10</sup>. In the present case, the patient had *PROC* levels of 1% upon admission.

Infants with severe congenital *PROC* deficiency typically present rapidly progressive purpura fulminans and DIC within hours of birth<sup>11</sup>. Purpura fulminans begins with red or purpuric lesions caused by application of mild pressure, which eventually forms painful, palpable black eschars. Most affected infants manifest white light reflexes and are congenitally blind due to thrombosis in the developing vitreal vein. On brain MRIs, many infants show evidence of prenatal arterial ischemic stroke or cerebral hemorrhage<sup>12</sup>. Large vessel thrombosis, including renal vein thrombosis, has been reported in some infants<sup>13</sup>. Purpura fulminans is triggered by infection, trauma, and even minor decreases in levels of therapeutic anticoagulants<sup>14,15</sup>. The present patient exhibited purpura fulminans, DIC, congenital blindness, and brain involvement, visual impairment and delayed development by 1 year of age.

Management of severe *PROC* deficiency includes an acute phase of replacement therapy with FFP or *PROC* concentrate, and an anticoagulant with warfarin or low-molecular-weight heparin as maintenance therapy<sup>16-18</sup>. The treatment reported here includes administration of FFP 4-5 times per week for 1 month, *PROC* concentrate, and oral enoxaparin after discharge.

Twenty cases of homozygous *PROC* deficiency presenting neonatal purpura fulminans have been reported worldwide since 1998. Most of these cases had a poor clinical outcome, including complete blindness (75%), severe neurological impairment (33%), or death within the first few days after birth (10%)<sup>19</sup>. Other major morbidities, such as renal failure, epilepsy, and impaired growth and development, have also been reported. Only 3 patients have been reported to not exhibit any major morbidity because of early onset of treatment and continuous therapy with *PROC* concentrate<sup>19,20</sup>.

Overall, the present study describes a case of severe *PROC* deficiency in a neonate that presented purpura fulminans, vitreous hemorrhage, and intracerebral hemorrhage. The diagnosis was confirmed by identification of a rare compound heterozygosity in *PROC*.

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