

## A Novel Protein C Mutation Causing Neonatal Purpura Fulminans

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**Background:** Neonatal purpura fulminans due to congenital protein C deficiency is a rare disorder. **Case characteristics:** A four-day-old neonate presented with multiple necrotic skin lesions with abnormal coagulation profile. **Intervention and outcome:** Skin lesions responded to repeated plasma transfusions but the neonate developed bilateral retinal detachment. A novel homozygous *PROC* gene mutation was noted in the neonate. **Message:** Molecular diagnosis and prenatal counseling in neonatal purpura fulminans are vital considering the poor outcome.

**Keywords:** Bleeding, Coagulation, Genetics, Neonate.

**P**urpura fulminans in neonates is a rapidly progressive thrombotic disorder manifesting as hemorrhagic skin infarction and disseminated intravascular coagulation. Congenital protein C deficiency presenting as neonatal purpura fulminans is an exceedingly rare condition with an incidence of 1 in 4 million [1]. It is caused by homozygous or compound heterozygous mutations in *PROC* gene with an autosomal recessive inheritance [2]. We report a case of novel homozygous *PROC* gene transversion mutation.

### CASE REPORT

A four-day-old male neonate was noted to have multiple necrotic skin lesions in thigh and sole of left leg on day 2 of life. He was third in birth order and born out of a term uneventful pregnancy to parents with second degree consanguinity. His vitals were stable and he was feeding well since birth. A new ecchymotic patch appeared over the entire right buttock on day 4. The lesions progressed rapidly with irregular central hemorrhagic necrosis (**Fig. 1**) surrounded by erythema. Investigation revealed thrombocytopenia (platelets count  $65 \times 10^9/L$ ), prolonged prothrombin time (19.2 s INR-2) and activated partial thromboplastin time (69 s) with normal hemoglobin and leucocyte counts. He had grossly elevated levels of D-dimer (8590 ng/mL) and fibrin degradation products (10 mcg/m; normal <5) suggestive of disseminated intravascular coagulation (DIC). Doppler of the cranial and renal vessels did not reveal any thrombosis. Computed tomogram of brain was normal. Ophthalmological examination revealed bilateral leucocoria and the B scan revealed bilateral vitreous hemorrhage and funnel shaped complete retinal detachment.

His elder female sibling had a similar clinical

presentation in the neonatal period with multiple cutaneous infarcts and DIC. She did not respond to treatment with fresh frozen plasma (FFP) transfusions and heparin therapy and died at one month of age. Considering this, a detailed laboratory work up to exclude congenital prothrombotic disorder was initiated. Protein C antigen (12.28%; normal 24-51) and activity (11%; normal 28-54) [3] estimated by chromogenic assays were found to be markedly decreased. Protein S and anti-thrombin III levels, were in the normal range. Parental protein C assay showed normal maternal levels. Father had low normal levels of protein C antigen (79%; normal 70-140) and decreased protein C activity (55%; normal 70-130).

Genomic DNA was isolated from the peripheral blood of the neonate and parents using Flexigene DNA extraction kit (Qiagen, Germany). Nine polymerase chain reaction (PCR) procedures were carried out for the 9 exons. The primers for exons 1, 2, 4/5, 7 and 8 were designed according to Lind, *et al.* [4]. The amplicons were sequenced using Big Dye Terminator cycle sequencing kit on an ABI 3130XL genetic analyzer (Applied Biosystems).

The causative molecular defect was a novel p.His253Tyr (C>T) transversion at the codon 253 of protein C gene. This missense mutation in exon 8 at codon 253 His>Tyr predicted a protein damaging serine protease domain to cause the protein C deficiency. This mutation was present in a homozygous state in our symptomatic neonate and in a heterozygous state in both asymptomatic parents (**Web Fig. 1**).

This severe congenital deficiency of Protein C was treated with multiple transfusions of FFP (20 mL/kg twice



**FIG.1** Multiple necrotic skin lesions (various stages) of purpura fulminans in a neonate.

daily) along with low molecular weight heparin (LMWH) during the first week. Protein C concentrate was not available. He was started on oral anticoagulant acenocoumarol from the second week. One week later, the estimated INR was 4. LMW heparin was stopped and acenocoumarol dose was titrated to attain target INR of 2.5-3.5. Skin lesions healed slowly over a month (**Fig. 1**) requiring FFP transfusions till the third week. Other deranged parameters during admission also normalized at discharge (platelet count  $450 \times 10^9/L$ , D-dimer-490 ng/mL, FDP-2 mcg/mL). The child is on follow up with long term anticoagulant therapy. No breakthrough thrombotic events were noted till now.

## DISCUSSION

Protein C is a vitamin K dependant anticoagulant that regulates thrombosis. Its role is especially important in the slow flowing venous circulation where there is prolonged exposure of procoagulant proteins and platelet phospholipids to the vessel wall with high risk of venous thrombosis. Neonatal purpura fulminans is a severe manifestation of homozygous Protein C deficiency usually presenting within few hours of birth with cutaneous infarcts and features of DIC. Thrombosis of cerebral vasculature and ophthalmologic complications including vitreous hemorrhage and retinal detachment resulting in partial or complete blindness are well known, with onset reported even in fetal period [5], and often not amenable to correction by postnatal management.

More than 230 unique mutations in *PROC* gene have been identified as on date [2]. Most of them are missense/nonsense mutations. Severe protein C deficiency often remains underdiagnosed as parents are asymptomatic and newborns have physiologically low levels of protein C along with difficulties in interpretation during the acute phase. It is possible that many of them die during the phase of DIC even before a diagnosis can be made.

Neonatal purpura fulminans is a medical emergency that warrants rapid normalization of plasma Protein C activity. One ml/kg of FFP may increase plasma protein C concentration by only 1 IU/dL [6]. Highly purified protein C concentrate (Ceprotin) is an alternative to repeated FFP transfusions [7]. After the acute phase, patients have to be transitioned to long term anticoagulation therapy. Breakthrough thrombotic events despite anticoagulation warrant exogenous protein C administration. Liver transplantation from living donors have been performed occasionally resulting in permanent cure [8,9].

Molecular diagnosis gives the parents an option of reliable prenatal diagnosis in the next pregnancy. This is crucial considering the poor prognosis and the need for multidisciplinary care to address the ophthalmological and neurological sequelae of this dreaded condition.

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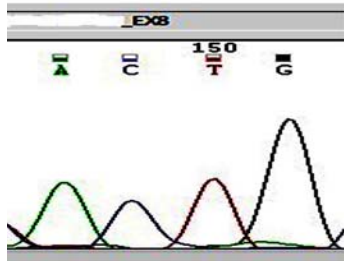
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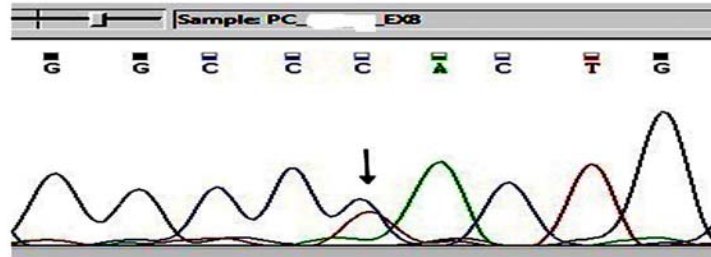
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His253Tyr  
(heterozygous)



C. Father: p His253Tyr  
(C>T, heterozygous)



WEB FIG. 1