A Novel Mutation in *G6PC3* Gene Associated Non-syndromic Severe Congenital Neutropenia

Severe congenital neutropenia (SCN) characterized by congenital neutropenia and maturation arrest in myeloid series is an orphan disorder with estimated prevalence of 6 per million [1]. Homozygous or compound heterozygous mutations in *G6PC3* gene, which encodes glucose-6-phosphatase enzyme, constitute around 2% of cases of SCN (MIM 612541) [2,3]. Till date, around 60 patients of this deficiency have been described in literature [4]. Majority of G6PC3 deficiency are syndromic and associated with prominent superficial veins, cardiac, genitourinary and renal disorders. Dursun syndrome has additional features involving non-myeloid hematopoietic cell lines, extra-hematologic features and pulmonary hypertension [1]. Recently, a non-syndromic phenotype has been described [4,5]. We describe a novel mutation associated with non-syndromic G6PC3 deficiency.

A five-month-old girl of southern Indian ethnicity, second born of second degree consanguineous marriage (birth weight 2600 gm) presented with multiple episodes of septicemia, pneumonia, acute gastroenteritis, and otitis media. She had a BCG scar and was developmentally normal. On examination, anthropometry revealed severe wasting (weight 4 kg, length 63 cms). There was no dysmorphism and hepato-splenomegaly.

Her hemoglobin records ranged from 8.5 to 10 g/dL with normal mean corpuscular volume, persistent neutropenia with absolute neutrophil count (ANC) between 100-1500/cmm and intermittent thrombocytopenia (nadir platelet count 1,10,000/cmm). Bone marrow examination revealed cellular marrow with maturation arrest at myelocyte stage; erythrocytes and megakaryocytes were normal. In view of recurrent infections with pancytopenia, differential diagnoses considered were primary immunodeficiency, inherited bone marrow failure syndrome and autoimmune neutropenia. Immunoglobulin profile was normal [IgG 889 (176-581) mg/dL, IgM 95 (24-102) mg/dL, IgA 99 (4-58) mg/dL]. HIV-ELISA and direct Coombs test were negative. Chromosome 22qdel by FISH was negative. T and NK cell subsets, CH50 screening test for complement pathway defect, dihydrorhodamine test were normal. Immunophenotyping of thymic function and anti-neutrophilic antibodies for auto-immune neutropenia were unavailable. Anti-tissue transglutaminase IgA and TORCH titres were normal. Chromosomal breakage studies and stool for fat globules were negative. Ultrasound was not suggestive of pancreatic fibrosis or renal anomaly. Targeted clinical exome sequencing by next generation sequencing was sent for congenital neutropenia. A homozygous single base pair deletion in exon 3 of the *G6PC3* gene variant c.372delC that results in a frameshift and premature truncation of the protein at codon 125 was detected. Echocardiography did not reveal any structural heart disease or pulmonary hypertension. Brainstem evoked response audiometry suggested mild hearing loss.

She was managed with antibiotic, antiviral and antifungal prophylaxis with granulocyte-colony stimulating factor (G-CSF) at 5µg/kg/day. After G-CSF, ANC improved to >1500/cmm without recurrence of any severe infection in next 6 months. Parents were counseled regarding the disease and were advised to avoid live vaccines. There was no donor available for bone marrow transplant. A trial off G-CSF was attempted at one year age. Parents were advised to use G-CSF if ANC fell below 500/cmm. At two years of age, ANC was between 1000-2000/cmm with no reported severe bacterial infections. She had normal development and growth, with weight and height presently between 10-25th centile. She did not require any further courses of G-CSF.

G6PC3 gene maps to 17q21.31, consists of six exons and encodes the G6PC3 protein. Homozygous missense mutations leading to frameshifts are described in four out of six reported non- syndromic cases. The largest number of missense mutations have been described in exon 6 [5]. Index child had deletion mutation in exon 3 which is a novel mutation in *G6PC3* responsible for SCN. *G6PC3* mutation causes G6PC3 deficiency affecting Glucose-6-phoshatase enzyme activity resulting in increased apoptosis of neutrophils leading to maturation arrest, subsequent neutropenia and diminished respiratory burst thereby accounting for both quantitative and qualitative defect in neutrophils [4]. A phenotypic heterogeneity and founder effect in Pakistani ethnicity has recently been described [4,5].

The typical hematological features described are severe neutropenia starting in early infancy and maturation arrest in myeloid lineage or myelokathexis. Dysfunctional neutrophilic activity, anemia, lymphopenia and intermittent thrombocytopenia are additional hematologic findings.

So far only six non-syndromic cases have been described in literature [1,4,5]. Index child did not have any of the above syndromic features. It remains unclear whether growth failure was primary or secondary to

recurrent infections. Similarly, sensorineural hearing loss is described in just three cases till date and has not been shown to be syndromically associated [4]. In the index child it may be attributed to recurrent ear infections. A prominent superficial venous pattern described in around two-third of the patient may not be present in infancy and becomes prominent with age. Non-syndromic presentation of G6PC3 deficiency should thus always be thought of in a child with isolated congenital neutropenia [1].

The genetic diagnosis is important in SCN as phenotypic variability exists; wherein most severe cases may require early bone marrow transplant (BMT), while rest may require G-CSF and infection prophylaxis awaiting a definitive BMT. Chronic G-CSF therapy has been inconsistently reported to be associated with myelodysplastic syndrome/myeloid leukemia and may not correct functional deficiency of neutrophil despite reversing neutropenia to a safer limit [6]. The use of G-CSF without clinical benefit should be avoided.

To conclude, SCN-4 should be considered in differential diagnosis of early onset severe neutropenia and indiscriminate use of G-CSF should be curtailed unless clinically indicated.

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An Untold Tale of Iron Deficiency Anemia

In India the prevalence of Iron deficiency anemia in children (6-59 months) is reported as 56% [1], most common by due to nutritional iron deficiency. Iron refractory iron deficiency anemia (IRIDA) is a rare genetic condition with autosomal recessive inheritance caused by mutation in *TMPRSS6* gene, located on chromosome 22 [2]. Suspicion of IRIDA usually occurs during pediatric age group when a child with iron deficiency anemia does not respond to standard therapy and has some suggestive markers of IRIDA.

The proband, a 9-year-old boy born of a consanguineous marriage, was diagnosed to have iron deficiency anemia at one year of age and treated with various iron preparation with inadequate response. Growth and development was adequate for the age. There was no icterus, bleeding manifestations, lymphadenopathy or hepatosplenomegaly. Blood examination revealed microcytic hypochromic anemia suggestive of iron deficiency anemia. His hemoglobin was 7g/dL; RBC count 5.4 × 10^9 L; mean corpuscular volume (MCV) 52 L, mean corpuscular hemoglobin (MCH), 15 pg/mL; mean corpuscular hemoglobin concentration (MCHC) 25.8%; red cell differential width (RDW) 19.6; serum iron, 1.2 µg/dL; Serum ferritin, 32 ng/mL; total iron binding capacity (TIBC) 352 µg/dL; and transfeerin saturation, 2.7%. Iron deficiency could not be explained from his diet, gastrointestinal losses or other symptoms. He was prescribed standard therapeutic doses of oral iron (6 mg/kg); however, response was not satisfactory (increase in hemoglobin Hb <0.5g in initial 4 weeks) despite adequate compliance. Further investigations revealed normal hemoglobin electro-phoresis and stool occult blood was tested negative. His younger brother was also detected to have anemia at 9 months of age and was on iron preparation with inadequate response.