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Novel STXBP2 Mutation Causing Familial Hemophagocytic Lymphohistiocytosis

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Familial Hemophagocytic Lymphohistiocytosis (FHL) is a rare autosomal recessive disorder. Diagnosis is established in presence of genetic mutation or positive family history in one of the siblings. Common genetic mutations associated with FHL are mutations in gene *PRF*-1 (also known as *FHL 2*), *UNC13D* (*FHL 3*) and *STX11* (*FHL 4*). Recently mutation in STXBP2 encoding syntaxin binding protein 2 (Munc 18 -2) has been found to be associated with FHL type 5. Here we describe the first reported Indian patient with homozygous mutation in *STX BP2* gene (c1697 G>A resulting in amino acid change p.G566D) causing FHL 5.

Key words: *Familial Hemophagocytic Lymphohistiocytosis, FHL 5, STXBP2 mutation.*

Familial hemophagocytic lymphohistiocytosis (FHL) is a genetically heterogeneous immune disorder characterized by widespread organ infiltration with activated macrophages and lymphocytes. The clinical course usually starts in infancy and is fatal unless treated with hematopoietic stem cell transplant. According to the guidelines of the Histiocyte Society, diagnosis of Hemophagocytic Lympho-histiocytosis (HLH) requires fulfillment of 5 out of 8 criteria – prolonged fever, splenomegaly, bicytopenia, elevated triglycerides/low fibrinogen, increased ferritin, hemophagocytosis in bone marrow, decreased NK cell cytotoxicity and increased soluble CD25 [1]. Diagnosis of FHL is established in presence of certain genetic mutations. Positive family history of affected siblings strongly suggests the diagnosis of FHL. Disease causing mutations in gene *PRF-1*encoding perforin, *UNC13d* encoding MUNC 13-4, and *STX11* encoding syntaxin11 have been identified in approximately 80% of cases. Recently mutation in *STX BP2* encoding syntaxin binding protein 2 (Munc 18 – 2) has been reported in a few patients. We report a patient with mutation in *STXBP2* gene from India.

CASE REPORT

A 28 day-old girl presented with fever, progressive abdominal distension and lethargy for seven days. She was the first child born to third degree consanguineous parents. During antenatal period mother gave history of fever and rash at fifth month of gestation. At 36 weeks of gestation mother had premature rupture of membranes and baby was delivered by caesarian section for

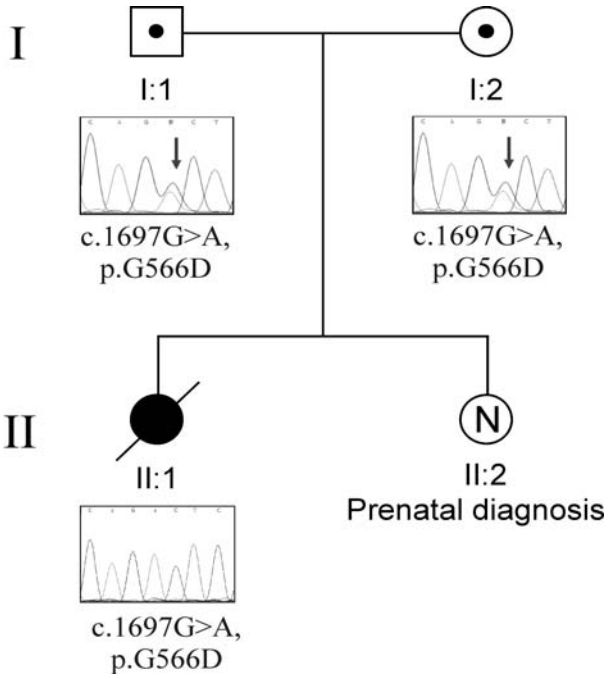

Fig. 1 *Chromatophreograms of the parents and the child highlighting the mutation in STXBP2 gene.*

complicated breech. Baby cried at birth and birth weight was 2.75 kg. Baby was admitted in NICU for suspected sepsis and received 5 days of antibiotics. After discharge she was well for 14 days. At 21 days of life she developed low grade fever, progressive abdominal distension and lethargy for which she was admitted elsewhere and treated with intravenous cefotaxime and amikacin. In view of decreasing hemoglobin and platelet count, she required transfusion with blood products and antibiotics were changed to vancomycin and meropenem. Bone marrow examination revealed haemophagocytosis. She was given one dose of IV Immunoglobulin and cyclosporine. Baby was brought to our hospital for further management.

Physical examination revealed pallor, liver 6 cm and spleen 4 cm palpable. Other system examination was unremarkable. Investigations revealed haemoglobin of 8.7gm%, platelet count 41,000/cu mm, total leukocyte count 27,200/cu mm, serum ferritin 13280 ng/mL, triglycerides 458 mg/dL and undetectable fibrinogen. Infection screen including blood culture, fungal culture, AFB culture, TORCH infection and parvo virus serology were all negative. A repeat bone marrow examination was done which showed increase in reticuloendothelial activity with haemophagocytosis and no malignant cells, thus fulfilling 6 out of 8 criteria for diagnosis of Hemophagocytic Lymphohistiocytosis. Extracted DNA of the child and the parents were sent for genetic analysis and she was started on dexamethasone. The child improved, organomegaly decreased and blood parameters normalized. Liver size at discharge was 2 cm, spleen 1cm. However 12 days after discharge she was readmitted with fever and poor feeding. Examination showed pallor and generalized skin mottling, cold peripheries and feeble pulses. Liver was palpable 3cm and spleen 2 cm. She was started on IV antibiotics, cyclosporine 6mg/kg/day was added after discussion with the hematologists. She developed refractory shock and succumbed later.

Initial DNA analysis report revealed negative mutation in *PRF-1, UNC13D* and *STX11* genes but later analysis revealed the new genetic mutation involving *STXBP2* gene. The child was found to be homozygous for the following novel mutation: c.1697G>A, resulting in amino acid change p.G566D. The same mutation was found at heterozygous state in both the parents. Mother is currently pregnant and prenatal DNA analysis of present fetus showed normal karyotype and negative for mutation in the *STXBP2* gene.

DISCUSSION

Four disease related genes have been identified in FHL. In a cohort study using samples from West Asian countries, mutations in already known genes (perforin, Munc13-4, and *STX11*) were identified in 80% FHL patients, while *STXBP2* mutation accounted for 10% and the cause remained unknown for the remaining 10% of FHL cases (2). *STXBP2* belongs to the Sec1/Mun18 family of regulatory proteins involved in the assembly and disassembly of SNARE (soluble *N*-ethylmaleimide sensitive factor attachment protein [SNAP] receptor) complexes and intracellular trafficking. *STXBP2* is required for degranulation of NK cell cytotoxic granules [3,4]. Mutation in *STXBP2* results in defective cytotoxic activity of NK cells.

Cetica, *et al.* [3] reported four patients with STXBP2 mutations, originating from Italy, England, Kuwait and Pakistan. Zur Stadt, *et al.* [5] reported 12 patients with *STXBP2* mutations from Turkey, Saudi Arabia, and Central Europe. Meeths, *et al.* reported 11 patients from Pakistan, Denmark, Netherlands, Norway and Russia and found that *STX BP2* mutation is associated with a spectrum of clinical symptoms other than those typically associated with HLH (colitis, bleeding disorders, and hypogammaglobulinemia). This may be a reflection of impaired expression and function of *STXBP2* in cells other than cytotoxic lymphocytes [6].

Our patient is the first reported Indian child having novel mutation c.1697G>A in *STXBP2* gene resulting in

amino acid change p.G566D and baby presented with typical manifestations of HLH. Early genetic testing is needed to confirm FHL as allogenic HCT is the only curative therapy. It further helps in testing of at risk relatives, carrier testing, genetic counseling and prenatal testing for pregnancies at risk if disease causing mutation in family are known. As in our case we did prenatal diagnosis for the second child which was negative for mutation in *STXBP2* gene.

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Infant with Type A Niemann Pick Disease and Undetectable Niemann Pick Cells in Bone Marrow

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Bone marrow aspiration is the preliminary investigation in Niemann Pick disease type A when enzyme assays and mutation studies are unavailable. We report an infant with typical phenotype and enzyme deficiency, but undetectable Niemann Pick cells in the bone marrow. A new mutation R542X in *SMPD* gene was also detected.

Key words: *Bone marrow, Diagnosis, Niemann pick disease type A, Storage cells.*

Niemann Pick Disease (NPD) is a lysosomal storage disorder caused by absence or deficiency of Acid Sphingomyelinase (ASM), leading to pathological accumulation of sphingomyelin and cholesterol in the monocyte-macrophage system. This is characterized by large lipid laden macrophages or Niemann Pick cells (NP cells) in various tissues. According to clinical presentation NPD is phenotypically classified as Type A (Classical infantile neuronopathic form), Type B (Non-neuronopathic visceral form) and Type C (Juvenile form). We report an infant with NPA, who despite having typical phenotype and enzyme deficiency, failed to display NP cells in the bone marrow.

CASE REPORT

A six month old boy presented with gradually progressive abdominal distension since late neonatal period. There was no history of persistent fever, vomiting, abnormal bowel movements, pallor, jaundice, bleeding, rash or additional swelling. Acquisition of all developmental milestones was delayed. Seizures and altered consciousness were absent. Antenatal and perinatal periods were normal. Birth was at