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CASE REPORT

A novel G6PC3 gene mutation in severe congenital neutropenia: pancytopenia and variable bone marrow phenotype can also be part of this syndrome

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Abstract

Glucose-6-phosphatase catalytic subunit 3 (G6PC3) deficiency is a newly described syndrome characterized by severe congenital neutropenia associated with multiple organ abnormalities including cardiac and urogenital malformations. The underlying pathophysiology of increased apoptosis of myeloid cells and of neutrophil dysfunction in G6PC3 deficiency involves disturbed glucose metabolism, increased endoplasmic reticulum stress and deficient protein folding. Here, we report a new case of G6PC3 deficiency caused by a novel homozygous G6PC3 gene mutation p.Trp59Arg. The patient showed pancytopenia and a variable bone marrow phenotype with maturation arrest and vacuolization in myeloid lineage cells and a normocellular marrow, respectively. She also showed persistent lymphopenia with low CD4 T- and CD19 B-cell counts. Lymphopenia and even pancytopenia as well as a variable bone marrow phenotype can be part of this syndrome. These clinical findings in a patient with chronic neutropenia should alert the clinician to consider a diagnosis of G6PC3 deficiency.

Key words congenital neutropenia; G6PC3; lymphopenia; variable bone marrow phenotype

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Congenital neutropenia syndromes represent a heterogenous group of disorders characterized by severe neutropenia and life-threatening infections early in life(1–3). Recently, remarkable progress has been made with regard to identification of the genetic defects causing severe congenital neutropenia. In addition to different genetic defects causing severe congenital neutropenia (HAX1, ELANE, WAS, GFI1), a novel syndromic congenital neutropenia was described caused by biallelic mutations in the glucose-6-phosphatase catalytic subunit 3 (G6PC3) gene(4–6). G6PC3 deficiency causes congenital neutropenia in combination with various developmental aberrations: patients with G6PC3 gene mutation are often characterized by intermittent thrombocytopenia, facial dysmorphism, increased visibility of superficial veins, heart and urogenital defects, inner ear hearing loss, growth retardation, primary pulmonary hypertension, cognitive impairment or endocrine abnormalities such as delayed puberty or hypothyroidism(7,8). G6PC3 deficiency leads to increased apoptosis of myeloid cells which could be linked to a defective glucose metabolism, increased endoplasmic reticulum stress, and unfolded protein response(7,9). Here, we report a new case of G6PC3 deficiency caused by a novel homozygous G6PC3 gene mutation p.Trp59Arg. The patient had a pancytopenia and variable bone marrow phenotype. She also had T- and B-cell lymphopenia as manifestation of a G6PC3 deficiency.

Case presentation

The patient is a 3-year-old girl, the second child of healthy first degree consanguineous parents. Her 6 months old sibling had a history of recurrent pneumonias and died because of cardiac defect and pneumonia. The rest of the family history was unremarkable. Our patient was born at term with

atrial septal defect and patent ductus arterious. She presented with osteomyelitis at 2 months followed by skin abscess on her leg. She had recurrent lung infections and otitis media. Her ear cultures were positive for candida albicans and klebsiella. In the following months, she developed progressive gastrointestinal symptoms especially chronic diarrhea. On physical examination, she had significant growth retardation, frontal bossing, depressed nasal bridge with upturned nose, and retrognatia. She had gingivitis, hepatomegaly, cutis laxa, and palmar erythema. Skin examination revealed a prominent superficial venous pattern on chest and abdomen.

Laboratory investigations during the first admission to our hospital demonstrated that her hemoglobin was 6 g/dL, white blood cell (WBC) count was 2.1×10^9/L, absolute neutrophil count (ANC) was 0.6×10^9/L, and platelet count was 89×10^9/L. ANC did not show a cyclic pattern in follow-up. Serum levels of iron, folic acid, and vitamin B12 were within normal ranges. Viral serology markers were negative. In the first bone marrow aspiration, there was a maturation arrest in cells of myeloid lineage with vacuolization. Her pancytopenia resolved overtime except for neutropenia and CD4+ T-cell and CD19+ B-cell lymphopenia. A second bone marrow aspiration revealed a normocellular marrow. The routine immunologic evaluation including serum immunoglobulin (Ig) G, IgA, IgM, IgE and vaccine responses were within normal limits. Peripheral blood mononuclear cells were isolated by Ficoll centrifugation. Lymphocytes were gated on the basis of their forward and side scatter properties by using FACS Calibur flow cytometry (Becton Dickinson). Peripheral blood $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, CD16+56, $TCR\alpha\beta^+$, and TCR $\gamma\delta^+$ cell counts were studied. The patient showed persistent CD4+ T-cell lymphopenia most often with cell counts of less than 500/mm^3 and persistent CD19+ B-cell lymphopenia (Table 1). Lymphocyte proliferation assays were normal. Nitroblue tetrazolium test for chronic granulomatous disease was normal. Echocardiography revealed atrial septal defect and pulmonary hypertension. Thorax CT of the lungs revealed bilateral infiltration and atelectasis. Her BCG scar was present and ppd result was 15 mm. Bronchoalveolar lavage was performed, acid-fast bacilli and cultures were negative and a few lipid laden macrophages were detected. INH with rifampicin prophylaxis was started. In BERA test, hearing loss was not detected. Abdominal ultrasonography showed hepatomegaly and bilateral cortical renal cysts. For chronic recurrent gastrointestinal symptoms especially for the chronic diarrhea, endoscopy and colonoscopy were performed, and no pathology compatible with inflammatory bowel disease was found. Molecular analysis for Pearson syndrome excluded mutation in Pearson gene. The treatment with subcutaneous G-CSF was started and the patient is currently receiving G-CSF (5 mcg/kg) therapy 2 days on a week. Her neutropenia showed good response to G-CSF. With G-CSF therapy, WBC and ANC remained between 2.1 to 12×10^9/L and 0.7 to 8×10^9/L, respectively. Based on the findings of congenital neutropenia, superficial venous pattern, cardiac and urinary defects, a diagnosis of G6PC3 deficiency was suspected.

Table 1 Immunologic work up of the patient

Hemoglobin values	Patient	Normal values
Hb (g/dL)	6–11	11–14
White cell count		
Leukocytes (cells/mm3)	2190–6340	4500–11000
Granulocytes (cells/mm3)	60–1200[1]	1800–7000
Lymphocytes (cells/mm3)	1050–3400	1700–6900
Eosinophils (cells/mm3)	0	0–400
Immunoglobulins		
IgG (mg/dl)	889	604–1941
IgA (mg/dl)	50	26–296
IgM (mg/dl)	130	71–235
IgE (KU/L)	<17	0–100
Lymphocyte population		
CD3+ T cells (/mm3)	1735–2575	900–4500
CD4+ T cells (/mm3)	260–436	500–2400
CD8+ T cells (/mm3)	1231–1542	300–1600
$TCR\alpha\beta^+/CD3^+$ (%)	84	85–95
TCR γδ+/CD3+(%)	32	5–15
CD19+ B cells (/mm3)	80–166	200–2100
CD16+56 NK cells (/mm3)	207–257	100–1000

[1]The upper range of ANC is not reflecting the use of G-CSF.

Two genomic DNAs have been extracted independently from patient's leukocytes using standard techniques. All exons including exon–intron boundaries of G6PC3 gene have been amplified each by PCR and sequenced directly. Mutational analyses were performed using BigDye Terminator v3.1 chemistry on an ABI3130 DNA analyzer (Applied Biosystems, Foster City, CA). The cDNA sequence variation has been described. Genetic analysis revealed a novel homozygous mutation p.Trp59Arg p.Trp59Arg (c.[175T>C]+[175T>C], p.[Trp59Arg]+[Trp59Arg]) in exon 1 of the G6PC3 gene confirming severe congenital neutropenia 4 (SCN4). Additional mutations in the SCN associated genes ELANE and HAX1 were excluded. The effect of p.Trp59Arg on protein function was predicted to be damaging or disease causing by using SNAP (http://www.broad.mit.edu/mpg/snap/), SIFT blink (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) *in vitro* algorithms.

Discussion

G6PC3 gene encodes glucose-6-phosphatase enzyme, which hydrolyzes glucose-6-phosphate to glucose in the final step of glycogenolysis. G6PC3 is a member of the glucose-6-phosphatase family, consisting of G6PC1, G6PC2, and G6PC3. G6PC1 is predominantly expressed in liver, gut, and kidney, G6PC2 is expressed in pancreas, and G6PC3 is ubiquitously expressed. G6PC3 is thought to act in a

complex with G6PT (glucose-6-phosphate translocase), they are located on the membrane of the endoplasmic reticulum. The transport of glucose-6-phosphate from cytosol to ER is facilitated by G6PT. Functional deficiency of the G6PC1/G6PT complex is associated with glycogen storage disease type Ib, whereas deficiency of the G6PC3/G6PT complex causes congenital neutropenia by increased apoptosis of neutrophils, defective glucose metabolism, increased endoplasmic reticulum stress, and unfolded protein response(7,9). Because of the glycosylation defects of target proteins in endoplasmic reticulum, Neutrophil nicotinamide adenine dinucleotide phosphate oxidase activity is also impaired in human G6PC3-deficient neutrophils(10). The glucose homeostasis appears to be of critical importance in neutrophil survival and function(9,11).

Immune problems of G6PC3 deficiency extend beyond neutropenia and neutrophil dysfunction(12). Lymphopenia and thymic hypoplasia were described in rare case reports. Begin *et al.* defined a patient with persistent low naive CD4+ T-cell lymphopenia suggesting a defect in thymic structure or function(13). Our patient also showed persistent CD4+ Tcell- and CD19+ B-cell lymphopenia. The mechanisms leading to lymphopenia are unclear. Lymphocytes appear relatively resistant to glucose deprivation. Boztug *et al (*9) showed that inhibition of glucose metabolism by 2-deoxyglucose did not increase apoptosis in CD3+ T lymphocytes as it gave rise in neutrophils in G6PC3 deficient patients. Lymphocyte subsets may be examined whether accompanying cellular immunodeficiency is suspected.

The pancytopenia, observed at admittance, might not be a reflection of the mutation, but rather by concurrent infection leading to anemia and thrombocytopenia. We cannot exclude finally that the patient has a concurrent disorder additionally. The disappearance of anemia and thrombocytopenia after treatment could reflect a kind of recovery of the bone marrow due to therapy. However, the anemia and thrombocytopenia in this patient could also have developed as a secondary feature due to bone marrow failure caused by the G6PC3 deficiency. The phenotypic spectrum of the condition is wide and variable and includes manifestations such as fluctuating patterns in all blood cell lines including neutropenia, thrombocytopenia, and anemia(14).

Bone marrow examination may show maturation arrest in the myeloid lineage but some patients with G6PC3 deficiency may have a hyper or normocellular bone marrow (15,16). A high neutrophil CXCR4 expression was shown in a G6PC3-deficient patient with myelokathexis. McDermott *et al.* proposed that in those patients, stress induces overexpression of neutrophil CXCR4, and this may contribute to neutropenia in G6PC3 deficiency(17). The bone marrow findings of our patient were variable ranging from maturation arrest to normocellular bone marrow. Even the same G6PC3 mutation can cause either maturation arrest or hypercellular or normocellular bone marrow. The reason underlying the variability of bone marrow phenotype is not clear. No genotype–phenotype correlation could be determined for bone marrow phenotype in patients with C6PC3 deficiency(15). G-CSF leads to increase of neutrophil numbers and prevents infections. G-CSF was shown to improve neutrophil function by stimulating glucose uptake in neutrophils in G6PC3 deficiency(18). Our patient was doing well with the use of G-CSF therapy twice a week.

The majority of the patients with G6PC3 deficiency defined in the literature revealed syndromic features comparable to our patient(19–23). However, more than 10% cases of G6PC3 deficiency could be non-syndromic(24). Therefore, G6PC3 deficiency should be considered as part of the differential diagnoses in any patient with unexplained congenital neutropenia.

The effect of a missense mutation like in this case acts mainly on the protein level i.e. by mistrafficking, misfolding, and misfunction of the protein. The validation of the disease causing relevance of the mutation found in our patient was *in vitro* confirmed by using predicting algorithms as standard procedure. (i) The prediction of the algorithms for the mutation p.Trp59Arg to be disease causative together with the fact of (ii) the apparent congruency of the presence of G6PC3 mutation with the clinical presentation of our patient strongly suggests for our diagnosis.

We conclude that the homozygous mutation p.Trp59Arg lead to G6PC3 deficiency which may present with lymphopenia and even pancytopenia with different phenotypes of bone marrow varying from myeloid maturation arrest to myelokathexis. These findings in a patient with chronic neutropenia should alert the clinician to consider a diagnosis of G6PC3 deficiency.

Conflict of interest statement

The authors in this manuscript do not have financial relationship with a commercial entity that has an interest in the subject of this manuscript.

This manuscript is not under simultaneous consideration by any other publication. All authors contributed the work, approved the final manuscript.

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