A Severe Congenital Neutropenia Type 4 Case (G6PC3 Mutation) Presented With Large Platelets in the Peripheral Smear

Meriç Kaymak Cihan, MD, Fatih Bolat, MD,† Hüseyin Onay, MD,‡ Ahmet Sarı, MD,§ Elif Ünver Korğalı, MD,§ Şükran Aslan, MD,§ Ceylan Cura, MD,§ and Dilara İçağasıoğlu, MD§*

Summary: Severe congenital neutropenia type 4 is a disorder of the hematopoietic system associated with mutations in the glucose-6-phosphatase catabolic 3 (G6PC3) gene. This disorder is characterized by neutropenia, congenital heart defects, urogenital malformations, and prominent superficial veins. To our knowledge, although intermittent thrombocytopenia is observed in this mutation, the coexistence of large thrombocytes is rarely seen. Here we present a case of severe congenital neutropenia type 4 with G6PC3 mutation and large platelets in the peripheral smear.

Key Words: severe congenital neutropenia type 4, G6PC3 mutation, large platelets

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Severe congenital neutropenia (SCN) is a hematologic disorder that is characterized by an absolute neutrophil count < 500 cells/mm^3.[1] The main clinical features of SCN type IV are congenital heart defects, prominent superficial veins, urogenital malformations, facial dysmorphism, and intermittent thrombocytopenia.[2] More severe and fatal forms of this syndrome are more likely to have primary pulmonary hypertension, leukopenia, and atrial septal defects.[3] Several phenotypic variants such as disrupted bone remodeling, hydronephrosis, and growth hormone deficiency were also reported.[4]

Glucose-6-phosphatase, an enzyme localized in the endoplasmic reticulum, catalyzes the hydrolysis of glucose-6-phosphate to glucose and inorganic phosphate.[5] In humans, there are 3 differentially expressed glucose-6-phosphatase catabolic genes (*G6PC1-3*). *G6PC1* is expressed mainly in the liver, the kidney, and the small intestine.[6–8] *G6PC2* is expressed only in pancreatic islet cells.[9,10] It plays role in glucose-dependent insulin secretion by controlling free glucose levels.[11] *G6PC3* is ubiquitously expressed.[12,13] Mutation of G6PC3 causes severe congenital neutropenia type 4 (SCN4). The neutrophils of these patients display enhanced endoplasmic reticulum stress and an increased rate of apoptosis.[2,14] The bone marrow morphology of SCN4 may show myeloid hypoplasia/hyperplasia, granulocyte maturation arrest at the myelocyte/promyelocyte stage, or no maturation arrest at granulocytes, myelokatexis, and dysplasia of granulocytes and megakaryocytes.[2,15,16] A definitive genotype-phenotype association of G6PC3 mutations has not been established yet.

Here we report a SCN4 case that presented with large platelets in the peripheral smear together with granulocyte and megakaryocyte dysplasia on bone marrow aspiration. Our patient is the first case in the literature whose *G6PC3* mutation is a homozygous *Y47X* (TAC > TAG) mutation with large platelets in the peripheral smear and granulocyte and megakaryocyte dysplasia on bone marrow aspiration.

CASE REPORT

A 1-day-old male infant was transferred to our neonatal intensive care unit for further management because of bilateral undescended testicles and prominent superficial veins (Fig. 1). He was born vaginally at 38 weeks of gestation with a birth weight of 2600 g and was the fourth child of healthy parents who are first cousins. The first male child died in the first 48 hours of life, the second female child was lost at the age of the 20 months. The etiologies of death of these children were unknown. The patient had a 6.5-year-old healthy brother. The physical examination was unremarkable except for bilateral undescended testicles and prominent superficial veins (Fig. 1). Laboratory data on admission were as follows: hemoglobin, 173 g/L; hematocrit, 48%; white blood cell count, 1.92×10^9/L; mean corpuscular volume, 109.4 fL; platelet count, 347×10^9/L; mean platelet volume (MPV), 12.8 fL (normal, 6 to 11 fL); and absolute neutrophil count, 0/mm^3. A peripheral blood smear revealed 44% monocytes, 56% lymphocytes, and large and giant platelets (Fig. 2E). Abdominal and cranial ultrasonographies were normal, but a scrotal ultrasound identified bilateral undescended testicles. Echocardiography was normal. The audiogram test was normal. Neutropenia and monocytosis persisted in repeated blood counts (Table 1). Toxoplasma

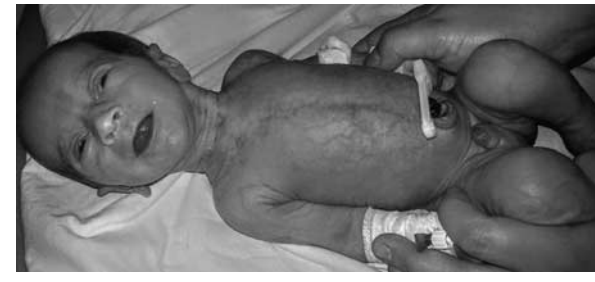

FIGURE 1. Prominent superficial veins of the patient.

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From the Departments of *Pediatrics, Division of Hematology and Oncology; †Pediatrics, Division of Neonatology; §Pediatrics, Faculty of Medicine, Cumhuriyet University, Sivas; and ‡Department of Medical Genetics, Faculty of Medicine, Ege University, İzmir, Turkey.
The authors declare no conflict of interest.
Reprints: Meriç Kaymak Cihan, MD, Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey (e-mail: merckaymak@gmail.com).

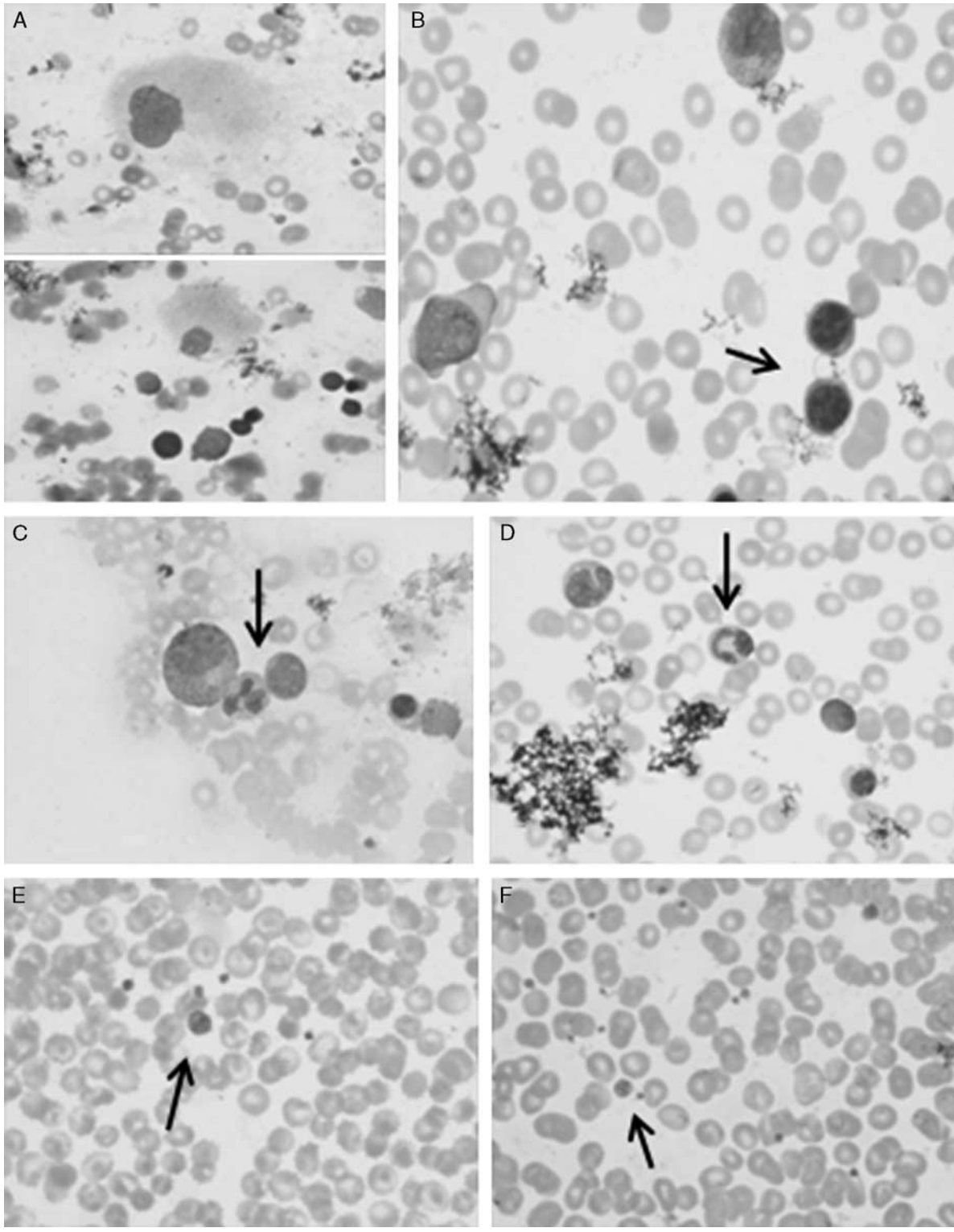

FIGURE 2. A, Bone marrow aspiration: single nucleated megakaryocyte (Giemsa, ×100). B, Bone marrow aspiration: arrow shows cytoplasmic bridge in ortchromatic erythroblasts (Giemsa, ×100). C and D, Bone marrow aspiration: arrow shows hypogranulation and hypolobulation in polymorphonuclear leukocytes (Giemsa, ×100). E, Patient's peripheral blood smear: arrow shows giant platelet (Giemsa, ×100). F, Mother's peripheral blood smear: arrow shows large platelet (Giemsa, ×100).

TABLE 1. Some Laboratory Values of the Patient

	September 11, 2014	November 17, 2014	December 04, 2014	December 30, 2014	March 30, 2015 (After G-CSF Therapy)
WBC (mm^3)	1290	6460	8310	3680	9590
Hb (g/L)	169	171	147	100	98
MCV (fL)	109	102	85	86,7	86
Platelets (mm^3)	111,000	340,000	638,000	256,000	378,000
ANC (mm^3)	0	129	0	441	4950
AMC (mm^3)	567	2455	3989	1690	1040
ALC (mm^3)	722	3876	4321	1325	3530
MPV (fL)	12.8	13.3	12	11.8	11.6
Peripheral smear	**44% monocytes, 56% lymphocytes, giant platelets +**	**60% lymphocytes, 38% monocytes, 2% neutrophils, giant platelets +**	**48% monocytes, 52% lymphocytes, giant platelets +**	**36% lymphocytes, 46% monocytes, 12% neutrophils (hypogranulation +), giant platelets**	**52% neutrophils, 10% monocytes, 38% lymphocytes, giant platelets +**
Immunoglobulins					
A (normal: 11-90 mg/dL)				12 mg/dL	
G (normal: 217-904 mg/dL)				581 mg/dL	
M (normal: 34-126 mg/dL)				38 mg/dL	
E: (normal: 0.76-7.31 IU/mL)				< 5 IU/mL	
Lymphocytes					
CD3 + (normal: 2400-8100/mm^3)				1000/mm^3	2200/mm^3
CD4 + (normal: 1000-5200/mm^3)				770/mm^3	1100/mm^3
CD8 + (normal: 600-3000/mm^3)				170/mm^3	1016/mm^3
CD19 + (normal: 500-3600/mm^3)				80/mm^3	434/mm^3
CD16 + 56(normal: 200-1800/mm^3)				120/mm^3	875/mm^3

ALC indicates the absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; Hb, hemoglobin; MCV, mean corpuscular volume; MPV, mean platelet volume; WBC, white blood cells.

IgM, rubella IgM, cytomegalovirus IgM, herpes virus type 1, and 2 IgM were negative. Bone marrow aspiration revealed 1.5% promyelocytes, 7% myelocytes, 0.5% metamyelocytes, 3% neutrophils, 2% bands, 10% lymphocytes, 13% monocytes, 4.5% promonocytes, and 55% normoblasts, with a myeloid/erythroid ratio of 0.25 (normal: 2 to 5.1). Maturation arrest of myeloid lineage was not observed. However, megakaryocyte and myeloid dysplasia characterized by single nucleated megakaryocytes, and hypogranulation in mature neutrophils was detected (Figs. 2A, C, D).

Cytoplasmic bridging in erythroblasts was also seen (Fig. 2B). Immunoglobulin A, G, and M levels were in the normal range; however, lymphocyte subsets of the patient were low according to his age (Table 1). Parental consanguinity, a history of sibling death, phenotypic features, and neutropenia findings were consistent with the diagnosis of SCN4. To confirm the diagnosis at the molecular level, the genomic DNA was isolated from a whole blood sample. Sequencing of the coding exons and the exon-intron boundaries was performed by the Illumina Miseq next-generation sequencing system. Mutation analysis revealed a homozygous *Y47X* (TAC > TAG) mutation, which was described before (Fig. 3). Blood counts and peripheral blood smear findings of the patient's mother, father, and brother were not compatible with neutropenia. On repeated analysis, the MPV level of the mother was found to be high (range, 11.8 to 12.7 fL) with large platelets in the peripheral blood smear (Fig. 2F).

Mutation analysis of the patient's father and mother showed a heterozygote carrier for *Y47X* mutation; however, his brother was normal. The in vitro bleeding time with a platelet function analyzer-100 (PFA-100) was in the normal range with collagen-epinephrine and collagen-adenosine diphosphate. Prophylactic trimethoprim-sulfamethoxazole 3 times weekly and daily fluconazole were started. Two months later, live vaccines were not administered because lymphocyte subsets of the patient were low according to the normal range (Table 1). Recombinant human granulocyte colony-stimulating factor (G-CSF) (5 mcg/kg/d) was initiated. During follow-up, the dose was decreased to 3 mcg/kg/d, maintaining total neutrophil counts > 1000/mm^3. After G-CSF was started, an increase in lymphocyte subset levels was seen at 5 months of age (Table 1). The Bacille Calmette-Guérin (BCG) vaccine was administered at 6 months of life. Severe infection was not observed in the patient so far. We stopped prophylactic trimethoprim-sulfamethoxazole and fluconazole. Currently, at the age of 6 months, he is followed up in our unit with a total neutrophil count > 1000/mm^3.

DISCUSSION

Severe congenital neutropenia type 4 is an unusual syndrome characterized by congenital heart defects, increased visibility of superficial veins, abnormal facial appearance, urogenital abnormalities, intermittent thrombocytopenia, failure to thrive, endocrine abnormalities, inner-ear hearing loss, and cutis laxa. Genetic research has identified the cause of syndromic forms of SCN4 with *G6PC3* mutation.[3] Non-syndromic SCN with *G6PC3* mutation has also been reported by Banka et al.[16] Superficial veins were the most

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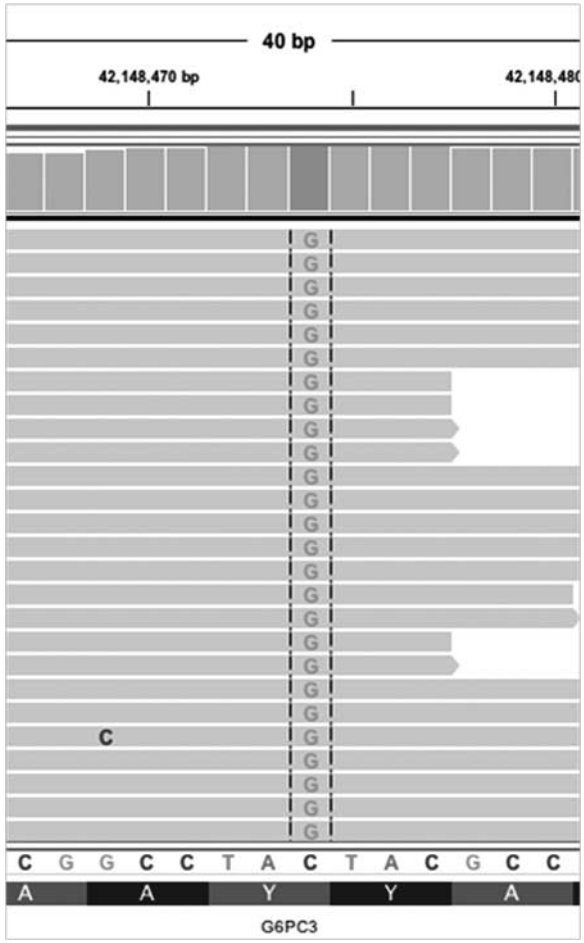

FIGURE 3. *Y47X* mutation detected in the *G6PC3* gene with the Illumina MiSeq system.

prominent clinical findings in patients with syndromic SCN4 as in our patient. We determined a homozygous mutation (*Y47X*) at the *G6PC3* gene in our patient. Previously, only 1 patient was shown to have a homozygote *Y47X* mutation.[2] This patient had prominent skin veins, hearing loss, and recurrent infections; however, neither congenital cardiovascular defect nor urogenital malformations were observed. Our patient lacked congenital heart defect or hearing loss, but had bilateral undescended testicles. Further investigations to show *G6PC3* genotype-phenotype are warranted.

Intermittent thrombocytopenia is the another feature of *G6PC3* mutation.[2,4,15,16] Our patient had mild thrombocytopenia at admission, and the thrombocytopenia improved within 24 hours. Although counts of thrombocytes were normal in range, his peripheral blood smear revealed giant platelets and his MPV was high, in accordance with large platelets on the peripheral smear of the patient's mother, and this finding continued for repeated examinations of blood smears. According to the information we have learned so far, macrothrombocytes with high MPV in the peripheral smear have been related to hematologic diseases and some metabolic diseases such as Bernard Solier syndrome, May-Hegglin anomaly and other *MYH-9*-related disorders, gray platelet syndrome, Swiss cheese platelet syndrome, Montreal platelet syndrome, and mucopolysaccharidoses.[17] To our knowledge, our report is the second one showing the coexistence of giant platelets with *G6PC3* mutation. McDermott et al[18] previously reported 2 siblings with G6PC3 mutation who had giant platelets in the peripheral smear. In addition, they had dysplastic features of megakaryocytes and myelokathexis in the bone marrow. We hypothesized that glucose homeostasis or the protein glycosylation defects in thrombocytes and neutrophils may be associated with possible large thrombocytes.[19] This finding may also explain the high turnover of thrombocytes. However, this hypothesis and the exact mechanism of large thrombocytes have to be tested in clinical trials. The presence of large platelets in case of the absence of thrombocytopenia did not cause functional anomaly for this patient (the in vitro bleeding time with PFA-100 was normal).

Parents of the patient were heterozygote carriers for the *Y47X* mutation. There is no information on whether heterozygote carriers are fully normal persons according to blood counts. Heterozygosity of this mutation might have caused the high MPV and large platelets on the peripheral smear of his mother. However, to explain this hypothesis, more investigations about carrier persons of this mutation have to be performed.

Bone marrow morphologies of *G6PC3* mutation are variable. It may be accompanied by maturation arrest of granulocytes at the promyelocyte/myelocyte stage.[2,4,15] In SCNs, dysplastic features of granulocytes can be seen.[20] In patients with *G6PC3* mutation myelokatexis, dysplasia of granulocytes and megakaryocytes is also reported.[15,16,18] Our patient had no maturation arrest of myeloid lineage in the bone marrow. However, we found that he had single nucleated megakaryocytes, hypolobulation, and hypogranulation at mature neutrophils.

Granulocyte colony stimulating factor (G-CSF) and prophylactic antibiotic treatment reduce the incidence of severe infections in patients with *G6PC3* mutation. The benefits of this indication are unclear.[2,4,15] Trimetoprim-sulphametaxasole, fluconasole, and G-CSF treatment were used prophylactically during the first 6 months of life in our patient. We did not notice any severe infection during this time period. The use of prophylactic antibiotics was discontinued when the total neutrophil count exceeded 1000/mm^3.

Naive T-cell deficiency was reported in some patients with *G6PC3* mutation.[15] In addition, in our patient, lymphocyte subsets were found to be low (Table 1). Thus, we did not administer live vaccines such as BCG at 2 months of age. The patient's lymphocytes increased gradually within 3 months. Then, the BCG vaccine was administered intradermally into the right deltoid muscle.

G6PC3 mutation is associated with increased morbidity and mortality, but data on the long-term outcome are limited. The patients can have long-term consequences, such as hypogonadism, psychomotor developmental delay, inflammatory bowel diseases, and cardiac dysrhythmias.[4,15] The monitoring of growth and neurological development of our patient was normal for his age.

Desplantes et al[15] described a patient with *G6PC3* mutation who developed acute myeloid leukemia at 14 years of age and required stem cell transplantation. Therefore, our patient was scheduled for bone marrow aspiration annually to detect progression to myelodysplastic syndrome or acute myeloid leukemia. Stem cell transplantation is not recommended routinely in the absence of malignancy.[2,4,15]

Our case was interesting because large thrombocytes together with G6PC3 mutation were observed. *G6PC3* mutation should be kept in mind in the differential diagnosis of prominent skin veins, congenital heart disease, urogenital anomalies such as undescended testicles, and neutropenia at complete blood count. It should not be forgotten that dysplastic features of megakaryocytes and granulocytes can be demonstrated in the bone marrow and the high MPV value with large platelets in the peripheral blood smear can be determined in patients with the *G6PC3* mutation. The causes of large thrombocytes in this mutation need to be further investigated.

REFERENCES

1. Klein C. Genetic defects in severe congenital neutropenia: emerging insights into life and death of human neutrophil granulocytes. *Annu Rev Immunol*. 2011;29:399–413.
2. Boztug K, Appaswamy G, Ashikov A, et al. A syndrome with congenital neutropenia and mutations in G6PC3. *N Engl J Med*. 2009;360:32–43.
3. Dursun A, Ozgul RK, Soydas A, et al. Familial pulmonary arterial hypertension, leucopenia, and atrial septal defect: a probable new familial syndrome with multisystem involvement. *Clin Dysmorphol*. 2009;18:19–23.
4. Boztug K, Rosenberg PS, Dorda M, et al. Extended spectrum of human glucose-6-phosphatase catalytic subunit 3 deficiency: novel genotypes and phenotypic variability in severe congenital neutropenia. *J Pediatr*. 2012;160:679–683.
5. Pan C-J, Lei K-J, Annabi B, et al. Transmembrane topology of glucose-6-phosphatase. *J Biol Chem*. 1998;273:6144–6148.
6. Mithieux G. New knowledge regarding glucose-6 phosphatase gene and protein and their roles in the regulation of glucose metabolism. *Eur J Endocrinol*. 1997;136:137–145.
7. Mithieux G, Rajas F, Gautier-Stein A. A novel role for glucose 6-phosphatase in the small intestine in the control of glucose homeostasis. *J Biol Chem*. 2004;279:44231–44234.
8. Pocai A, Obici S, Schwartz GJ, et al. A brain-liver circuit regulates glucose homeostasis. *Cell Metab*. 2005;1:53–61.
9. Arden SD, Zahn T, Steegers S, et al. Molecular cloning of a pancreatic islet-specific glucose-6-phosphatase catalytic subunit-related protein. *Diabetes*. 1999;48:531–542.
10. Martin CC, Bischof LJ, Bergman B, et al. Cloning and characterization of the human and rat islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) genes. *J Biol Chem*. 2001;276:25197–25207.
11. Petrolonis AJ, Yang Q, Tummino PJ, et al. Enzymatic characterization of the pancreatic islet-specific glucose-6-phosphatase-related protein (IGRP). *J Biol Chem*. 2004;279:13976–13983.
12. Martin CC, Oeser JK, Svitek CA, et al. Identification and characterization of a human cDNA and gene encoding a ubiquitously expressed glucose-6-phosphatase catalytic subunit-related protein. *J Mol Endocrinol*. 2002;29:205–222.
13. Guionie O, Clottes E, Stafford K, et al. Identification and characterisation of a new human glucose-6-phosphatase isoform. *FEBS Lett*. 2003;551:159–164.
14. Aróstegui JI, de Toledo JS, Pascal M, et al. A novel G6PC3 homozygous 1-bp deletion as a cause of severe congenital neutropenia. *Blood*. 2009;114:1718–1719.
15. Desplantes C, Fremond ML, Beaupain B, et al. Clinical spectrum and long-term follow-up of 14 cases with G6PC3 mutations from the French severe congenital neutropenia registry. *Orph J Rare Dis*. 2014;9:183.
16. Banka S, Wynn R, Newman WG. Variability of bone marrow morphology in G6PC3 mutations: is there a genotype-phenotype correlation or age-dependent relationship? *Am J Hematol*. 2011;86:235–237.
17. Bussel J. Disorders of platelets. In: Lanskowsky P, ed. *Manual of Pediatric Hematology and Oncology*, 5th ed. San Diego, CA: Elsevier; 2011:321–377.
18. McDermott DH, De Ravin SS, Jun HS, et al. Severe congenital neutropenia resulting from G6PC3 deficiency with increased neutrophil CXCR4 expression and myelokathexis. *Blood*. 2010;116:2793–2802.
19. Hayee B, Antonopoulos A, Murphy EJ, et al. G6PC3 mutations are associated with a major defect of glycosylation: a novel mechanism for neutrophil dysfunction. *Glycobiology*. 2011;21:914–924.
20. Olcay L, Ünal Ş, Öztürk A, et al. Granulocytic and non granulocytic lineages in children with congenital neutropenia and their non neutropenic parents: biochemical, functional, morphological and genetic evaluation. *Haematologica*. 2012;97(suppl):36–37.