J Clin Immunol (2013) 33:1403–1406
DOI 10.1007/s10875-013-9945-7

BRIEF COMMUNICATION

A Novel Homozygous Mutation in *G6PC3* Presenting as Cyclic Neutropenia and Severe Congenital Neutropenia in the Same Family

Abdullah A. Alangari · Abdulrahman Alsultan · Mohamed Elfaki Osman · Shamsa Anazi · Fowzan S. Alkuraya

Received: 18 June 2013 / Accepted: 30 September 2013 / Published online: 9 October 2013

Abstract
Purpose Patients with autosomal recessive cyclic neutropenia have no known causative genetic defect yet.
Methods Autozygosity mapping on two branches of an extended multiplex consanguineous family presenting with cyclic neutropenia or severe congenital neutropenia to look for candidate gene, followed by candidate gene selection and sequencing.
Results A single autozygous interval on Chr17:33,901,938-45,675,414 that is exclusively shared by the affected members was identified. This interval spans 11.8 Mb and contains 30 genes. Review of these genes highlighted *G6PC3* as the most likely candidate given its known role in neutrophil biology. Direct sequencing revealed a novel homozygous mutation (NM_138387.3, c.974T>G, p.Leu325Arg). Two of our patients had associated congenital defects that are known to occur in patients with *G6PC3* mutations, including congenital heart disease and intermittent thrombocytopenia.
Conclusion Biallelic *G6PC3* defects should be considered in patients with autosomal recessive cyclic neutropenia, especially those with typical associated congenital defects.

A. A. Alangari (✉) · A. Alsultan · M. E. Osman
Department of Pediatrics, College of Medicine, King Saud University, P.O. Box 2925, Riyadh 11461, Saudi Arabia
e-mail: aangari@ksu.edu.sa

S. Anazi · F. S. Alkuraya
Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

F. S. Alkuraya
Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia

Keywords Cyclic neutropenia · severe congenital neutropenia · autozygosity mapping · G6PC3 · congenital heart disease · thrombocytopenia

Introduction

Cyclic neutropenia (CyN) is defined as an absolute neutrophil count (ANC) less than 0.5×10^9/L for at least 3 to 5 days per approximately 21 day cycles [1]. The only known genetic defect in patients with CyN so far is mutated neutrophil elastase (NE) gene (*ELANE,* formerly known as *ELA2*) located on chromosome 19p13.3 [2, 3]. This condition is inherited in an autosomal dominant fashion.

Glucose 6-phosphatase catalytic subunit-3 (G6PC3) is a ubiquitously expressed enzyme that is involved in the stability of the endoplasmic reticulum (ER) by maintaining cellular energy homeostasis through recycling of ER glucose to the cytoplasm [4]. It is encoded by the gene *G6PC3* located on chromosome 17q21. Biallelic mutations in *G6PC3* result in an autosomal recessive disease of severe congenital neutropenia (SCN), known as SCN type 4. SCN is defined as an ANC of less than 0.5×10^9/L present with invasive bacterial infections. It is usually associated with maturation arrest at the promyelocyte/myelocyte stage [5, 6]. The neutropenia in G6PC3 deficiency is of variable severity and is commonly associated with other congenital abnormalities including cardiac anomalies, urogenital malformations, and venous angiectasia [7]. This neutropenia is thought to result from cellular apoptosis secondary to increasing ER stress [5].

G6PC3 deficiency has not been described to cause CyN. Here we describe an extended multiplex Saudi family in

which mutated *G6PC3* is associated with phenotypes consistent with CyN in some members and SCN in others.

Materials and Methods

Human Subjects

All patients and their unaffected first-degree relatives (parents and siblings) were enrolled in an IRB-approved protocol and a written informed consent was obtained. Venous blood was collected in EDTA tubes for DNA extraction and subsequent genetic studies.

Autozygosity Analysis

DNA from all enrolled subjects was subjected to genomewide genotyping on the Axiom SNP Chip platform (Affymetrix) following the manufacturer's protocol. Genotypes were used to determine the autozygome for each individual essentially as described before [8]. An autozygous interval that is exclusively shared by the affected members was considered a candidate locus for a founder homozygous disease-causing mutation and the genes within the locus were reviewed for candidacy based on published data.

Sequencing

The entire coding region and flanking intronic sequences of *G6PC3* were amplified by especially designed primers (primer sequence and PCR conditions are available upon request) followed by bidirectional Sanger sequencing. Resulting variants were crosschecked against a local database of 250 Saudi exomes, as well as the Exome Variant Server (EVS) and other available SNP databases. The variant that was absent in the aforementioned databases was further screened in a panel of 96 normal Saudi controls by direct sequencing.

Results

Clinical Report

Multiple affected individuals belonging to two distantly related branches of a single extended multiplex consanguineous family (Fig. 1a) presented with clinical features consistent with CyN in one branch and SCN in the other as described below.

Patient IV:9 is a 12 years old boy who presented with recurrent 2–3 days episodes of aphthous stomatitis associated with abdominal pain and vomiting and sometimes fever up to 40 °C every 3–4 weeks since he was 1.5 years old. These symptoms were immediately preceded by episodes of neutropenia (when measures were obtained) that may reach as low as 0.07×10^9/L (Fig. 2). His absolute monocyte count (AMC) may have a picture of opposite oscillation to neutrophils, but is most of the time$\geq0.5\times10^9$/L. More recently he had history of intermittent thrombocytopenia down to 100×10^9/L that did not correlate with his neutrophil count changes. His RBCs count was consistently normal. His neutrophil killing function by dihydrorhodamine (DHR) oxidative burst assay was normal. Bone marrow aspiration, done when he had a normal peripheral neutrophil count, showed active trilineage hematopoiesis with no evidence of granulocytic maturation arrest. There was marked clinical improvement after starting granulocyte colony stimulating factor (GCSF) (Fig. 2). No history of major or life threatening infections. He was diagnosed to have asthma, bicuspid aortic valve, indirect inguinal hernia, and recently started to complain of large joints arthralgia and long bones pain especially after exercise.

Patient IV:8 is a 10 years old girl who has symptoms, like her brother, of recurrent aphthous stomatitis, abdominal pain, and fever. Each episode lasts for 2–3 days and recurs every month. It is typically preceded by a drop in ANC that reached 0.21×10^9/L during a month of close observation (Fig. 2). No history of recurrent infections. She has intermittent thrombocytopenia down to 74×10^9/L. She is also asthmatic and was diagnosed with type II atrial septal defect (ASD). No history of hernias.

Patient IV:6 was born at term and developed respiratory distress at 10 h of age that rapidly progressed to septic shock. This was accompanied by severe neutropenia. He died shortly afterwards on the second day of life.

Patient IV:3 is a 9 year old girl who presented with frequent episodes of fever that started in the second month of life. She had pneumonia, gastroenteritis, otitis media, gingivitis, and abscesses at vaccination sites. She also has mild intermittent asthma. She has chronic neutropenia with ANC ranging between $0.11–0.6\times10^9$/L (Fig. 2). Lymphocyte subsets enumeration and DHR oxidative burst assay were normal. Bone marrow aspiration while neutropenic (ANC$=0.27\times10^9$/L) showed active granulopoiesis with no maturation arrest. There was no evidence of myelodysplastic syndrome or acute meylogenous leukemia. Bone marrow biopsy showed normal cellularity. No structural defects were detected.

Patient IV:1 is a 2 year old boy who presented at 6 months of age with penile abscess. Culture was positive for *Pseudomonas aeruginosa*. His ANC dropped to zero within 1 week and was$\leq0.18\times10^9$/L for another 2 weeks until GCSF was started. Bone marrow aspiration while having severe neutropenia (ANC$=0.02\times10^9$/L) showed active granulopoiesis with no maturation arrest. Bone marrow biopsy was not done. No structural defects were detected. Figure 2 shows the trend of his ANC and AMC while not on GCSF and in the absence of infection. There is no family history of myelodysplasitc syndromes or acute myeloid leukemia.

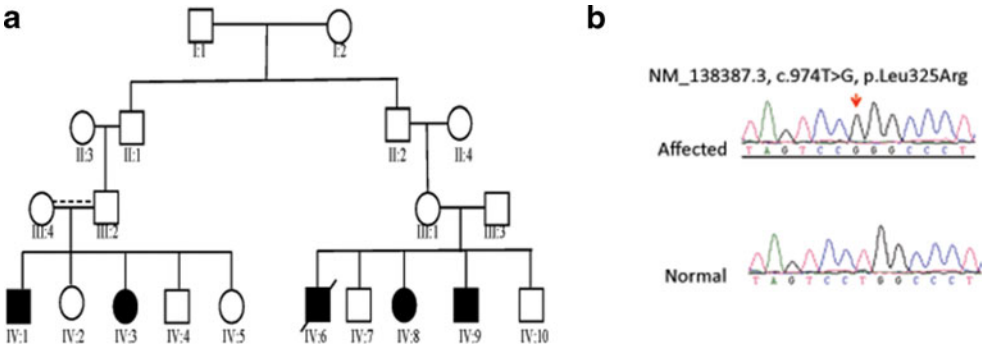

Fig. 1 **a**. Pedigree of an extended consanguineous multiplex family segregating both SCN and CyN phenotypes. **b**. Sequence chromatogram of *G6PC3* showing a homozygous mutation. A control is shown for comparison

Identification of a Novel *G6PC3* Homozygous Mutation in a Family with CyN

Consistent with our hypothesis that all affected members of this extended consanguineous family have a homozygous causal mutation that resides on a shared ancestral haplotype, we have identified a single autozygous interval on Chr17:33, 901,938-45,675,414 that is exclusively shared by the affected members. This interval spans 11.8 Mb and contains 30 genes. Review of these genes highlighted *G6PC3* as the most likely candidate given its known role in neutrophil biology. Indeed, direct sequencing revealed a novel homozygous mutation (NM_138387.3, c.974 T>G, p.Leu325Arg) (Fig. 1b) that was absent in 250 in-house Saudi exomes, EVS, dbSNP as well as 96 Saudi controls by direct sequencing.

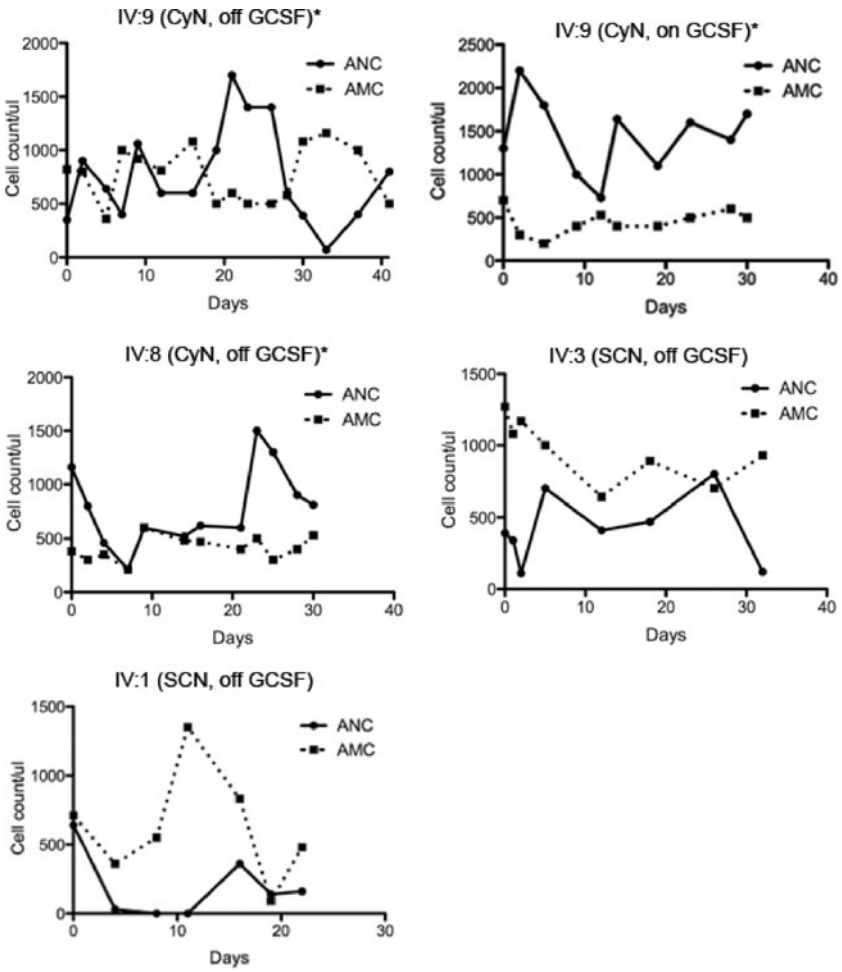

Fig. 2 Graphs demonstrating a sequence of absolute neutrophil counts and absolute monocyte counts of the affected family members over time. (*) Indicates patients who had other stigmata of G6PC3 deficiency; patient IV:9 has thrombocytopenia, bicuspid aortic valve, inguinal hernia, arthralgia; patient IV:8 has thrombocytopenia and type II ASD

Discussion

Patients IV:9 and IV:8 have a clinical phenotype compatible with CyN, although different from the classically described phenotype in patients with NE deficiency, in that it is inherited in an autosomal recessive pattern and it has associated clinical features not found in that condition [1, 3]. Consistent with the original description of patients with CyN, [9] our patients have their cycles roughly every 3 to 4 weeks. Patients with SCN also have variability in their neutrophil counts, including patients with G6PC3 deficiency, but it is most of the time below 0.5×10^9/L as observed in patients IV:3 and IV:1 [7, 10]. This suggests that CyN and SCN could represent a wide range spectrum [11]. The associated features and structural defects that our patients with CyN phenotype have were all reported in the past in patients with G6PC3 deficiency [7, 10].

Patient IV:9 had opposite oscillatory changes of his monocytes as compared to his neutrophil count (Fig. 2). This feature has been demonstrated in patients with *ELANE* mutations [1]. However, in contrast to many patients with NE deficiency he did not have oscillation of other hematopoietic cell lines like platelets or reticulocytes count within the same cycle [9]. On the other hand, our patients with SCN phenotype demonstrated compensatory monocytosis, a feature that is frequently observed in patients with SCN [11]. The normal bone marrow picture in our patients is a typical feature of cyclic neutropenia. However, some patients with G6PC3 deficiency and SCN were shown to have normal or even hypercellular marrow [12, 13]. This was proposed to be secondary to increased neutrophils expression of *CXCR4*, a chemokine receptor with neutrophil bone marrow retention properties [14].

The coexistence of SCN and CyN in the same kindred was reported in patients with *ELANE* mutations [15]. In addition, similar to patients with *ELANE* mutations, GCSF did not change the cycling pattern in patient IV:9; rather, it has shifted the curve upwards [3].

In conclusion, we have shown for the first time that a *G6PC3* homozygous mutation resulted in a phenotype that is compatible with CyN in addition to the classical phenotype of SCN. Therefore, mutations in that gene should be considered in patients with CyN presenting with an autosomal recessive pattern of inheritance or with associated defects reported in G6PC3 deficiency.

Acknowledgments The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-190

Conflict of interest None of the authors has any potential financial conflict of interest related to this manuscript.

References

1. Haurie C, Dale DC, Mackey MC. Cyclical neutropenia and other periodic hematological disorders: a review of mechanisms and mathematical models. Blood. 1998;92(8):2629–40.
2. Horwitz M, Benson KF, Person RE, Aprikyan AG, Dale DC. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. Nat Genet. 1999;23(4):433–6. doi:10.1038/70544.
3. Horwitz MS, Duan Z, Korkmaz B, Lee HH, Mealiffe ME, Salipante SJ. Neutrophil elastase in cyclic and severe congenital neutropenia. Blood. 2007;109(5):1817–24. doi:10.1182/blood-2006-08-019166.
4. Jun HS, Lee YM, Cheung YY, McDermott DH, Murphy PM, De Ravin SS, et al. Lack of glucose recycling between endoplasmic reticulum and cytoplasm underlies cellular dysfunction in glucose-6-phosphatase-beta-deficient neutrophils in a congenital neutropenia syndrome. Blood. 2010;116(15):2783–92. doi:10.1182/blood-2009-12-258491.
5. 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. doi:10.1146/annurev-immunol-030409-101259.
6. Ward AC, Dale DC. Genetic and molecular diagnosis of severe congenital neutropenia. Curr Opin Hematol. 2009;16(1):9–13. doi:10.1097/MOH.0b013e32831952de.
7. Boztug K, Appaswamy G, Ashikov A, Schäffer AA, Salzer U, Diestelhorst J, et al. A syndrome with congenital neutropenia and mutations in G6PC3. N Engl J Med. 2009;360(1):32–43. doi:10.1056/NEJMoa0805051.
8. Alkuraya FS. Discovery of rare homozygous mutations from studies of consanguineous pedigrees. Current protocols in human genetics / editorial board, Jonathan L Haines [et al]. 2012;Chapter 6:Unit6 12. doi:10.1002/0471142905.hg0612s75.
9. Haurie C, Dale DC, Mackey MC. Occurrence of periodic oscillations in the differential blood counts of congenital, idiopathic, and cyclical neutropenic patients before and during treatment with G-CSF. Exp Hematol. 1999;27(3):401–9.
10. Boztug K, Rosenberg PS, Dorda M, Banka S, Moulton T, Curtin J, 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(4):679–683.e2. doi:10.1016/j.jpeds.2011.09.019.
11. Donadieu J, Fenneteau O, Beaupain B, Mahlaoui N, Chantelot CB. Congenital neutropenia: diagnosis, molecular bases and patient management. Orphanet J Rare Dis. 2011;6:26. doi:10.1186/1750-1172-6-26.
12. Smith BN, Evans C, Ali A, Ancliff PJ, Hayee Bah, Segal AW, et al. Phenotypic heterogeneity and evidence of a founder effect associated with G6PC3 mutations in patients with severe congenital neutropenia. Br J Haematol. 2012;158(1):146–9.
13. 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(2):235–7. doi:10.1002/ajh.21930.
14. McDermott DH, De Ravin SS, Jun HS, Liu Q, Priel DA, Noel P, et al. Severe congenital neutropenia resulting from G6PC3 deficiency with increased neutrophil CXCR4 expression and myelokathexis. Blood. 2010;116(15):2793–802. doi:10.1182/blood-2010-01-265942.
15. Newburger PE, Pindyck TN, Zhu Z, Bolyard AA, Aprikyan AAG, Dale DC, et al. Cyclic neutropenia and severe congenital neutropenia in patients with a shared ELANE mutation and paternal haplotype: evidence for phenotype determination by modifying genes. Pediatr Blood Cancer. 2010;55(2):314–7. doi:10.1002/pbc.22537.