Extended Spectrum of Human Glucose-6-Phosphatase Catalytic Subunit 3 Deficiency: Novel Genotypes and Phenotypic Variability in Severe Congenital Neutropenia

Kaan Boztug, MD[1,2,3], Philip S. Rosenberg, PhD[5], Marie Dorda[3], Siddharth Banka, MD[7], Thomas Moulton[8], Julie Curtin, PhD[9], Nima Rezaei, MD[10,11], John Corns, DO[13], Jeffrey W. Innis, MD, PhD[14], Zekai Avci, MD[16], Hung Chi Tran, MD[17], Isabelle Pellier, MD[18], Paolo Pierani, MD[19], Rachel Fruge[20], Nima Parvaneh, MD[11], Setareh Mamishi, MD[12], Rajen Mody, MD[15], Phil Darbyshire, MD[22], Jayashree Motwani, MD[22], Jennie Murray[23], George R. Buchanan, MD[21], William G. Newman, MD, PhD[7], Blanche P. Alter, MD, MPH[6], Laurence A. Boxer, MD[15], Jean Donadieu, MD, PhD[24], Karl Welte, MD[4], and Christoph Klein, MD, PhD[3,25]

Objective To delineate the phenotypic and molecular spectrum of patients with a syndromic variant of severe congenital neutropenia (SCN) due to mutations in the gene encoding glucose-6-phosphatase catalytic subunit 3 (G6PC3).

Study design Patients with syndromic SCN were characterized for associated malformations and referred to us for *G6PC3* mutational analysis.

Results In a cohort of 31 patients with syndromic SCN, we identified 16 patients with G6PC3 deficiency including 11 patients with novel biallelic mutations. We show that nonhematologic features of G6PC3 deficiency are good predictive indicators for mutations in G6PC3. Additionally, we demonstrate genetic variability in this disease and define novel features such as growth hormone deficiency, genital malformations, disrupted bone remodeling, and abnormalities of the integument. *G6PC3* mutations may be associated with hydronephrosis or facial dysmorphism. The risk of transition to myelodysplastic syndrome/acute myeloid leukemia may be lower than in other genetically defined SCN subgroups.

Conclusions The phenotypic and molecular spectrum in G6PC3 deficiency is wider than previously appreciated. The risk of transition to myelodysplastic syndrome or acute myeloid leukemia may be lower in G6PC3 deficiency compared with other subgroups of SCN. *(J Pediatr 2012;160:679-83)*.

Severe congenital neutropenia (SCN) comprises a heterogeneous group of disorders commonly characterized by lack of mature neutrophils, increased susceptibility to bacterial infections and risk of malignant transformation to myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML).[1] Mutations in the *neutrophil elastase* (*ELANE*, formerly termed *ELA2* [Mendelian Inheritance in Man (MIM) 202700])[2] or *HS1-associated protein X1* (*HAX1*)[3] [MIM 610738] genes have been shown to cause autosomal dominant and autosomal recessive variants of the disease, respectively. More rarely, SCN can be caused by mutations in the *growth factor independence-1*[4] [MIM 600871] or activating mutations in the *Wiskott-Aldrich syndrome* [MIM 301000][5] genes.

AML	Acute myeloid leukemia
CN	Congenital neutropenia
ELANE	*Neutrophil elastase*
G6PC3	Glucose-6-phosphatase catalytic subunit 3
HAX1	*HS1-associated protein X1*
HSCT	Hematopoietic stem cell transplantation
MDS	Myelodysplastic syndrome
SCN	Severe congenital neutropenia

From the [1]Research Center for Molecular Medicine of the Austrian Academy of Sciences (CeMM); [2]Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria; [3]Department of Pediatric Hematology/Oncology and; [4]Department of Molecular Hematopoiesis, Hannover Medical School, Hannover, Germany; [5]Biostatistics Branch and; [6]Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD; [7]Genetic Medicine, St Mary's Hospital, Manchester Academic Health Sciences Centre, Manchester, United Kingdom; [8]Department of Pediatric Hematology/Oncology, Bronx Lebanon Hospital, Bronx, NY; [9]Department of Hematology, the Children's Hospital at Westmead, Westmead, Sydney, NSW, Australia; [10]Molecular Immunology Research Center and Department of Immunology, School of Medicine; [11]Research Center for Immunodeficiencies, Children's Medical Center and; [12]Infectious Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran; [13]Department of Pediatric Hematology/Oncology, Nationwide Children's Hospital, Columbus, OH; [14]Division of Pediatric Genetics, University of Michigan; [15]Department of Pediatrics, University of Michigan Hospital, Ann Arbor, MI; [16]Department of Pediatric Hematology, Kecioren Research and Training Hospital, Kecioren, Ankara, Turkey; [17]Division of Hematology/Oncology, Department of Pediatrics, Children's Hospital Los Angeles, Los Angeles, CA; [18]Centre Hospitalier Universitaire Angers, Angers, France; [19]Division of Pediatric Hematology/Oncology, Institute of Mother and Child Health, G. Salesi Women's and Children's Hospital, Polytechnic University of Marche, Italy; [20]The Center for Cancer and Blood Disorders, Children's Medical Center; [21]Division of Hematology/Oncology, Department of Pediatrics, The University of Texas Southwestern Medical Center, Dallas, TX; [22]Department of Haematology, Birmingham Children's Hospital, Birmingham, United Kingdom; [23]South-East Scotland Clinical Genetics Services, Western General Hospital, Edinburgh, United Kingdom; [24]Service d'Hémato Oncologie Pédiatrique, Hôpital Trousseau, Paris, France; and [25]University Children's Hospital, Ludwig Maximilians University, Munich, Germany

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Recently, we defined a novel complex syndrome (SCN4 [MIM 612541]) associating SCN and developmental aberrations such as congenital heart defects, urogenital malformations, and increased visibility of superficial veins. Mutations in the gene encoding glucose-6-phosphatase catalytic subunit 3 (*G6PC3*) were identified as the molecular cause of this disorder.[6] Similar to other genetic subgroups of SCN, G6PC3 deficiency is associated with increased myeloid cell apoptosis, which could be linked to activation of the "unfolded protein response" and activation of glycogen synthase kinase 3β and degradation of antiapoptotic MCL-1.[6] Recent work has suggested that impaired recycling of glucose from the endoplasmic reticulum to the cytoplasm may underlie neutrophil dysfunction in G6PC3 deficiency.[7] G6PC3 deficiency also may be associated with primary pulmonary hypertension, cognitive impairment, or endocrine abnormalities such as delayed puberty or hypothyroidism.[8-12] In view of the phenotypic variability, we hypothesized that the full phenotypic spectrum of this condition has not yet been appreciated.

Patients with mutations in the *ELANE/ELA2* or *HAX1* gene have a high incidence of MDS/AML.[13-15] However, the association of SCN4 with the risk of MDS/AML has not yet been studied systematically.

Here we report 31 patients with syndromic SCN and identify 16 patients with G6PC3 deficiency, expanding the clinical and molecular spectrum of this rare disorder. We also report novel phenotypic features of SCN4 and estimate the risk of MDS/AML in this disorder.

Methods

We investigated patients with syndromic congenital neutropenia (CN) referred to us from various collaborating institutions for mutation analysis of *G6PC3*. Clinical data were collected via the referring clinicians. We defined syndromic CN as congenital neutropenia associated with at least one additional nonhematologic aberration, such as increased superficial venous marking, congenital heart defect, and/or urogenital abnormalities.

The study was approved by the institutional review boards of Hannover Medical School, University of Manchester (06138), and NHS ethics committee (06/Q1406/52). All material from patients and healthy donors was obtained with informed assent/consent in accordance with current European regulations.

PCR and Sequencing Analysis of *G6PC3*

G6PC3 was sequenced in 23 patients with a complex variant of CN in Hannover and in 8 patients with SCN in Manchester. Polymerase chain reaction amplification of all exons and exon-intron boundaries of the human *G6PC3* gene was performed as described previously.[6] Subsequent DNA sequencing was performed on an ABI3130XL genetic analyzer (Applied Biosystems, Darmstadt, Germany).

Statistical Analysis

We estimated the crude rate of MDS/AML in 16 patients from this group in whom we identified *G6PC3* mutations as well as in our 12 previously published patients with G6PC3 deficiency.[6] We compared this with the corresponding rate in 374 patients enrolled in the Severe Chronic Neutropenia International Registry[13] for whom long-term follow-up data are available. The latter comparison group consists of patients with disease causing mutations in *ELANE* or *HAX1* known to be associated with increased risk of leukemogenesis, as well as patients for whom the causative genetic defect and therefore the associated leukemia predisposition are unknown. The most recent follow-up data available for the Severe Chronic Neutropenia International Registry was used for this study.[15] We compared the rate of MDS/AML in patients with biallelic *G6CP3* mutations versus all patients with clinically defined SCN using data on the numbers of MDS/AML and the person-years at risk in each group, and the exact binomial test for the number of events in the smaller group of patients with G6PC3 deficiency.[16] The null hypothesis for the test was that the rate of MDS/AML in patients with *G6PC3* mutations is the same as the rate in all patients with clinically defined SCN.

Results

In this study, we investigated 31 patients with a syndromic variant of CN for mutations in *G6PC3*. We identified 14 (61%) of 23 patients with biallelic *G6PC3* mutations (ie, the same or different mutations on both alleles) in the German cohort (patients [P] 1-14 in **Table I**) and 2 (25%) of 8 in the British cohort (P15 and P16), giving a total of 16 (43%) of 31 new patients with G6PC3 deficiency. The 16 new patients with G6PC3 deficiency represent the single largest SCN4 cohort (**Figure** and **Table II**; available at www.jpeds.com); 5 (31.2%) patients had compound heterozygous mutations, and 11 (68.8%) were homozygous. We identified 11 novel mutations, including frame-shift deletions (P6, P7, P10, P11, and P15) and a single nucleotide insertion (P5). The other 5 novel mutations in this study were missense (P7, P12, P13, P14, and P16).

All 16 patients with G6PC3 deficiency had at least one of the characteristic syndromic features of G6PC3 deficiency such as increased superficial venous marking, congenital heart defect, and/or urogenital abnormalities (**Table I**). Prominent superficial venous pattern is the most consistent and striking nonhematologic feature of G6PC3 deficiency, and was noted in 14 of 16 patients in this cohort (**Table I**).

In addition to known clinical features of G6PC3 deficiency,[6] several novel characteristics were noted. One patient had a hypoplastic left ventricle (P15), a cardiac feature that has not been reported previously in SCN4. Similarly, the spectrum of urogenital aberrations is wider than previously appreciated,[6] including ambiguous genitalia (P7) and micropenis in males (P3 and 4) and discontinuous labia majora and minora in females (P1). Hydronephrosis has been previously reported in 1 patient,[12] and here we have identified 2 additional patients with congenital hydronephrosis (P7, P12), supporting this association.