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A novel syndrome with congenital neutropenia caused by mutations in *G6PC3*

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Author contributions

K.B. designed and performed most of the experiments and identified the first *G6PC3* mutation, wrote the initial draft of the manuscript and critically participated in all further revisions of the manuscript. G.A. performed immunoblot analyses and caspase activation assays. A.A. performed phosphatase assays. A.A.S. performed linkage analysis computations, chose microsatellite markers to genotype in the linkage region and wrote parts of the manuscript. U.S. provided laboratory resources for genotyping and tested whether control individuals carry the R253H mutation. J.D. performed sequencing of candidate genes and sequenced patients for *ELA2*, *HAX1*, and *G6PC3* mutations. M.G. sequenced *ELA2*, *HAX1*, *G6PC3* and *CSFR3* in patient samples. G.B. performed electron microscopy studies. J.L.-G. performed candidate gene sequencing. F.N. helped with cloning of retroviral vectors. A.-K.G. performed E. coli killing assays. M.M., J.G., C.Kr., T.P., I.P., C.B.-C., N.R., K.M. and N.I.-H. obtained clinical samples and provided clinical data. H.B. and R.G.-S. supervised A.A. and were involved in critical discussions. C.Z. cared for patients and collected data in the SCN patient registry. B.G. provided laboratory resources and assisted A.A.S. K.W. provided resources for the SCN registry and significant help to carry out this study. C.Kl. designed and initiated the study, directed the course of investigations, provided laboratory and financial resources, and wrote the manuscript together with K.B.

K.B., A.A.S. and C.Kl. analyzed the data in this study. C.Kl. decided to publish this manuscript and vouches for the data.

Disclosure

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Abstract

Background—Severe congenital neutropenia (SCN) is characterized by early onset of severe bacterial infections due to a paucity of mature neutrophils. There is also an increased risk of leukemia. The genetic causes of SCN are unknown in many patients.

Methods—Genome-wide genotyping and linkage analysis were performed on two consanguineous pedigrees with a total of five children affected with SCN. Candidate genes from the linkage interval were sequenced. Functional assays and reconstitution experiments were carried out.

Results—All index patients had susceptibility to bacterial infections and myeloid maturation arrest in the bone marrow; some had structural heart defects and venous angiectasia on the trunk and extremities. Linkage analysis of the two index families yielded a combined multipoint LOD score of 5.74 on a linkage interval on chromosome 17q21. Sequencing of the candidate gene *glucose-6-phosphatase catalytic subunit 3 (G6PC3)* revealed a homozygous missense mutation in exon 6 in all affected children in the two families, abrogating enzymatic activity of Glucose-6-phosphatase. Neutrophils and fibroblasts of patients had increased susceptibility to apoptosis. Myeloid cells showed evidence of increased endoplasmic reticulum stress and increased activity of GSK3 β . We identified seven additional, unrelated SCN patients with syndromic features and distinct biallelic mutations in *G6PC3*.

Conclusions—Defective function of G6PC3 defines a novel SCN syndrome associated with cardiac and urogenital malformations.

Syndromes associated with congenital neutropenia are a heterogeneous group of disorders ^{1, 2}. Severe congenital neutropenia was described more than 50 years ago by Kostmann ^{3, 4}. In these syndromes, the paucity of neutrophils in peripheral blood causes life-threatening bacterial infections early in life. Most patients respond to recombinant human granulocyte-stimulating factor (rh-G-CSF), which increases peripheral neutrophil counts and decreases the frequency and severity of infections ⁵. Nonetheless, patients may remain at risk for both infectious complications and the development of clonal disorders of hematopoiesis such as myelodysplastic syndrome or acute myeloid leukemia ⁶.

Considerable progress has been made in identifying the molecular defects that cause congenital neutropenia ^{7, 8}. Many patients with severe congenital or cyclic neutropenia have a heterozygous mutation in the *neutrophil elastase (ELA2)* gene ^{9–11}. We recently identified homozygous mutations in *HAX1* in a subgroup of patients with autosomal recessive severe congenital neutropenia ¹². In addition, mutations in *WAS* ^{13, 14} and *GFII* ¹⁵ have been associated with a phenotype resembling Kostmann's syndrome. In many patients with congenital neutropenia, however, the underlying molecular cause remains unknown. Despite recent insights into the role of apoptosis ^{12, 16, 17} the mechanisms of neutropenia and the risk of leukemia in severe congenital neutropenia are incompletely understood.

Here we report a syndrome, which to our knowledge has not been previously recognized, associating severe congenital neutropenia with extra-hematopoietic features, which is caused by bi-allelic mutations in the gene encoding the glucose-6-phosphate catalytic subunit 3 (*G6PC3*).

Methods

Patients and controls

Blood and bone marrow samples from patients and healthy individuals were taken upon informed consent. The study was approved by the institutional review board at Hannover Medical School.

Genome-wide linkage analysis

We genotyped microsatellite markers in a whole genome scan for family SCN-I. Equipment and protocols for genotyping were as described previously¹². The genetic linkage analysis was done using a combination of quantitative and qualitative syllogisms. The quantitative decisions were made using LOD scores and optimal recombination fractions computed with the software Superlink^{18, 19}. For LOD score computations, we modeled neutropenia as a fully penetrant autosomal recessive disease with no phenocopies and disease allele frequency 0.001. The Marshfield map²⁰ was used to select usefully positioned markers for fine mapping. For details, see Supplementary Information.

Amplification and sequence analysis of the *G6PC3* gene

Exons and flanking intron-exon boundaries from candidate genes were PCR-amplified and analyzed using an ABI Prism 3130 DNA Sequencer and the DNA Sequencing Analysis software version 3.4 (Applied Biosystems, Foster City, CA, USA) and Sequencer version 3.4.1 (Gene Codes Corporation, Ann Arbor, USA). For primer sequences and details on the restriction length polymorphism analysis to analyze the frequency of the R253H mutation in healthy controls, refer to Supplementary Methods.

Isolation of early myeloid progenitors from bone marrow

Promyelocytes were sorted by FACS as described previously with minor modifications¹⁷.

Real-Time PCR analysis

Gene expression analysis of *G6PC3* and *HSPA5/Bip/Grp78* was performed using a Lightcycles 2.0 (Roche). See Supplement for details.

Determination of enzymatic activity of wildtype and mutant *G6PC3*

The complete open reading frames of wild type and mutant *G6PC3* were PCR amplified and cloned in pYES-cup1 (modified from pYES-NT (Invitrogen) as described in²¹ and expressed in *Saccharomyces cerevisiae*. The 100,000 g microsomal fraction was assayed for glucose-6-phosphate (G6P) hydrolysis to glucose by addition of [¹⁴C]G6P (MP Biomedicals). Released [¹⁴C]glucose was separated from G6P by anion exchange and measured in the eluate by liquid scintillation.

Immunoblot analyses

Whole cell lysates from primary granulocytes were separated by SDS-PAGE, blotted and stained with antibodies against phospho-Mcl-1 (Ser159/Thr163), total GSK3 β phospho-GSK3 β (Ser9) (all from Cell Signaling/New England Biolabs, Frankfurt am Main, Germany), Bip/Grp78 (BD Biosciences, Heidelberg, Germany) and GAPDH (Santa Cruz Biotechnologies, Heidelberg, Germany). Please refer to the supplement for further information.

Electron microscopy

Bone marrow samples from patients and healthy controls were subjected to hypotonic lysis. Fixation and electron microscopy were performed as described previously²².

Retroviral gene transfer experiments

The human *G6PC3* cDNA was cloned into a bicistronic retroviral MMP vector²³ containing murine *cd24* as a marker gene. RD114-pseudotyped retroviral particles were generated by tripartite transfection of MMP-based vectors together with the envelope plasmid and the packaging plasmid mPD.old.gag/pol into the HEK 293T cell line. Transduction of CD34+ cells and myeloid differentiation was performed as described previously¹².

Apoptosis assays

Apoptosis in peripheral blood neutrophils or in vitro differentiated myeloid cells was induced using TNF- α (50 ng/ml), thapsigargin (10 μ M) or tunicamycin (5 μ g/ml; all from Sigma) and assessed by Annexin-V (Invitrogen)/propidium iodide (Sigma) staining. In fibroblasts, apoptosis was induced using 5mM dithiothreitol (Roche). Caspase 3/7 activation was assessed as described previously¹². For details, refer to Supplementary information.

Results

Clinical Findings

Table 1 lists the main features of the five patients we studied. The siblings P1 and P2, born to consanguineous parents of Aramean descent, presented with neonatal sepsis. their extended pedigree is denoted SCN-I (Suppl. Fig. 1). Further workup in their first year of life revealed severe neutropenia, apparently congenital, with a paucity of mature neutrophils in peripheral blood and bone marrow. Phenotypically, bone marrow smears showed a pathognomonic maturation arrest at the stage of promyelocytes/myelocytes (Fig. 1a, b and Suppl. Table 1). Erythrocyte counts were normal. Platelet counts in P1 ranged from 73,000–425,000, while P2 had normal platelet counts. Both patients had unusually prominent subcutaneous veins and/or venous angiectasia (Fig 1c); P1 had atrial septal defect (ASD) type II, and P2 had cor triatriatum (Fig. 1d) and hepatosplenomegaly. Genealogical investigations revealed that the SCN-I pedigree could be extended to include two additional sibships each having one child also affected by severe congenital neutropenia and ASD-II (Patients #3 and 4, Suppl. Fig. 1). We also identified a child with severe congenital neutropenia in a second consanguineous pedigree (SCN-II) from the same ethnic background (Patient #5). All patients received recombinant human G-CSF (rh-G-CSF) and responded with an increase in peripheral neutrophil counts.

Genetic Studies

Mutations in both *ELA2*¹⁰ and *HAXI*¹² were excluded in all five index patients. Genetic linkage analysis gave statistical evidence that the gene mutated in SCN-I is located on chromosome 17q21 between D17S1299 (36.2Mb, 62.0cM) and D17S1290 (53.7Mb, 82.0cM) (Fig. 2a and Supplementary Results). We carried out a series of fine mapping steps in SCN-I and SCN-II and were able to genotype an additional 13 microsatellite markers between D17S1299 and D17S1290 in SCN-I, and 11 of these in SCN-II. Supplementary Table 2 shows single-marker LOD scores. Assuming that the same gene is mutated in all five affected children, the maximal linkage interval spanned from D17S1789 (39.1Mb, 63.1cM) to D17S791 (42.2Mb, 64.2cM). Using D17S932, D17S950, and D17S806, the peak multipoint LOD score in SCN-I alone was 4.98, and the peak two-pedigree multipoint LOD score was 5.74.

Several candidate genes were identified in the SCN-I linkage interval (Supplementary Table 3). Of these, *G6PC3*, encoding the glucose-6-phosphatase catalytic subunit-3, and located in the narrowest possible linkage interval, was a plausible candidate, because abnormal glucose metabolism has been implicated in neutropenia. in glycogen storage disease type Ib patients²⁴. DNA sequencing revealed a homozygous missense mutation in exon 6 of the *G6PC3* gene (c. G758A, p. R253H) (Fig. 2b). This mutation was found in all four affected

children in SCN-I and in the affected child in SCN-II. All parents were heterozygous at this position, confirming autosomal recessive inheritance of a germline missense mutation. With restriction site analysis, the G6PC3^{R253H} allele was not found in 192 healthy central European individuals. An *in silico* sequence analysis using SIFT²⁵ predicted that the probability of this mutation being benign was 0.01; analysis with PolyPhen²⁶ predicted that the R253H mutation is probably damaging to protein function, as expected since R253 is conserved in multiple species including mammals, amphibians, bony fish, and insects.

Functional Studies

Enzymatic Activity of G6PC3^{R253H}—G6PC3^{wildtype} and G6PC3^{R253H} were expressed in *Saccharomyces cerevisiae*. Microsomes were isolated from yeast transfected with G6PC3^{wildtype} or G6PC3^{R253H}, and assayed for phosphatase activity. G6PC3^{wildtype} hydrolyzed glucose-6-phosphate and the universal substrate p-Nitrophenylphosphate (pNPP), as demonstrated by radioactive (Fig. 2c) and spectrometric (Suppl. Fig. 3) assays, respectively. In contrast, the level of enzymatic activity of mutant G6PC3^{R253H} did not exceed the phosphatase level in yeast transfected with an empty vector.

Apoptosis: Similar to patients with mutations in *ELA2*¹⁰ or *HAX1*¹², peripheral blood neutrophils had an increased rate of spontaneous apoptosis in all five patients tested. Apoptosis was also markedly accelerated in patients' neutrophils after induction with either TNF- α (Fig. 3a) or tunicamycin (data not shown), as assessed by Annexin-V staining and a test for caspase-3/7 activation, respectively (see also Suppl. Fig. 4). Since *G6PC3* is a ubiquitously expressed gene and since the phenotype of our patients was not restricted to the hematopoietic system, we tested non-hematopoietic cells for susceptibility to apoptosis. Skin fibroblasts from G6PC3-deficient patients displayed an increased susceptibility to apoptosis following DTT-induced stress to the endoplasmic reticulum (Fig. 3b).

To provide further evidence that this novel form of SCN is caused by mutations in *G6PC3*, we performed reconstitution experiments to correct premature apoptosis in myeloid cells. CD34⁺ hematopoietic stem cells from two patients were isolated and transduced with retroviral constructs containing either the wildtype *G6PC3* cDNA sequence and murine *CD24* as a reporter gene (MMP-G6PC3-mCD24) or the reporter gene only (MMP-mCD24). Upon *in vitro* differentiation in the presence of recombinant human G-CSF and GM-CSF, cells were exposed to tunicamycin to induce apoptosis and analyzed by flow cytometry by gating on mCD24-positive cells. In control-transduced cells from a patient, exposure to tunicamycin induced a high degree of apoptosis (29.99% Annexin-V positive +4.63% AnnexinV/propidium iodide double positive cells), whereas in G6PC3-transduced cells, apoptosis was reduced (17.86% + 1.90%) (Fig 3c; Suppl. Fig. 5). We tested the function of neutrophils in G6PC3-deficient neutrophils. Both phagosomal lysis of *E. coli* and the oxidative burst were comparable to neutrophils from healthy control individuals (Suppl. Fig. 6).

Endoplasmic reticulum stress: Endoplasmic reticulum (ER) stress and the unfolded protein response have been linked to the pathophysiology of aberrant organogenesis²⁷, including structural heart defects²⁸, and congenital neutropenia caused by mutations in *neutrophil elastase* (*ELA2*)^{17, 29}. We therefore sought evidence for increased endoplasmic reticulum stress in our patients. Transmission electron microscopy of bone marrow cells from all four G6PC3-deficient patients analyzed showed an enlarged rough ER in myeloid progenitor cells as compared with such cells from a healthy individual (Fig. 4a, 4b), consistent with increased ER stress (Suppl. Fig. 7 shows electron microscopy in other patients). BiP mRNA, another marker of increased ER stress, was measured by RT-PCR in bone marrow promyelocytes isolated by flow cytometry. The BiP mRNA level was increased in promyelocytes from both patients tested compared to promyelocytes from healthy individuals (Fig. 4c).

GSK3 β : Recently, a signalling circuit linking glucose, GSK3 β , and Mcl1 has been established^{30, 31}. GSK3 β controls glycogen metabolism, Wnt-signalling, and apoptosis³². Mcl1, an anti-apoptotic member of the Bcl2 family, is involved in maintenance of neutrophil viability³³. GSK3 β phosphorylates Mcl1, thus facilitating its degradation via the proteasome³⁰. We performed Western blot studies to estimate the levels of GSK3 β , BiP, and Mcl1 proteins in neutrophils exposed to the ER stress-inducing agent tunicamycin. We observed increased levels of BiP (Fig. 4e), an increase in the enzymatically active dephosphorylated form of GSK3 β (Fig. 4d and Suppl. Fig. 8), and increased phosphorylation of Mcl1 in neutrophils from all patients examined (n= 2) (Fig. 4e). To investigate whether intracellular glucose deprivation causes de-phosphorylation of GSK3 β , we inhibited glucose metabolism in neutrophils from two healthy individuals using 2-deoxyglucose (2DG). Treatment with 2DG induced dephosphorylation of GSK3 β (Fig. 4f) and increased apoptosis of neutrophils (Fig. 4g), whereas CD3-positive T lymphocytes were resistant to the effects of 2DG (Fig. 4g).

G6PC3 mutations in Other Patients: We assessed the frequency and variety of *G6PC3* mutations in a cohort of patients with genetically unclassified severe congenital neutropenia. 104 such patients were examined for mutations in *G6PC3*, and seven of these had distinctive biallelic mutations in *G6PC3* (Table 1). The mutations include nonsense mutations (Y47X, Y48X) that would destroy the functional parts of the protein, even if the truncated mRNA is translated rather than being subjected to nonsense mediated decay. The three additional missense mutations were predicted to be deleterious by SIFT²² analysis, with probabilities of being benign of 0.03 for L185P, 0.00 for G262R and 0.00 for G260R, respectively. None of these additional patients with *G6PC3* mutations had mutations in *ELA2* or *HAX1*, suggesting that these three genetic defects are distinct variants of severe congenital neutropenia. Of note, none of the *G6PC3*-deficient patients suffered from hypoglycemia or lactic acidosis (Supplementary Table 4), as seen in patients with glycogen storage disorders.

A comprehensive clinical review of all 12 *G6PC3*-deficient patients revealed variability of clinical features. Of the 12 patients, eight had various cardiac malformations, and ten had a phenotype of unusually prominent subcutaneous veins and/or venous angiectasia (Fig. 1 and Supplementary Fig. 2). Five patients had urogenital malformations, including cryptorchidism and urachal fistula. Two patients had inner ear hearing loss. While two patients showed growth delay, no consistent dysmorphic features were noted (Table 1).

Discussion

We describe a novel congenital neutropenia syndrome caused by biallelic mutations in *G6PC3*. Similar to patients with mutations in *HAX1*¹² or *ELA2*¹⁶, *G6PC3*-deficient patients had an evidence of myeloid maturation arrest and increased apoptosis in peripheral neutrophils. Of twelve patients, 8 had structural heart defects (e.g., atrial septal defect type II, cor triatriatum, pulmonary stenosis) and 5 had urogenital defects (e.g., cryptorchidism, urachal fistula). In most patients, angiectasia of superficial veins was prominent. The broad spectrum of developmental aberrations may depend on factors other than the mutant *G6PC3*. Perhaps increased susceptibility to apoptosis also affects cardiac or urogenital development in *G6PC3*-deficient patients. There is clinical variation in other neutropenia syndromes such as Cohen syndrome and cartilage hair hypoplasia, even though the two syndromes are genetically homogeneous⁸. All patients in our cohort responded to treatment with rh-G-CSF, and to date no patient has developed a clonal hematopoietic disorder.

Three human genes mediating glucose-6-phosphatase activity have been discovered to date: *G6PC1*, *G6PC2*, and *G6PC3*. *G6PC1*, the classical glucose-6-phosphatase expressed in liver/kidney/small intestine catalyzes the hydrolysis of glucose-6-phosphate, an essential step in the

gluconeogenic and glycogenolytic pathways. Patients without G6PC1 activity suffer from glycogen storage disease type Ia³⁴. *G6PC2* is expressed uniquely in pancreas islet cells^{35, 36} and may be involved in glucose-dependent insulin secretion by controlling free glucose levels³⁷. Statistically significant associations between noncoding polymorphisms in or near *G6PC2* and the risk of diabetes have been shown^{38, 39}. In contrast to *G6PC1* and *G6PC2*, *G6PC3* is ubiquitously expressed^{40, 41}. Glucose-6-phosphate is transported to the endoplasmic reticulum via a glucose-6-phosphate transporter (G6PT)⁴². Although the stoichiometry and topological relationships between the catalytic subunits of glucose-6-phosphatase and G6PT remain unclear, their functional link has been recognized^{42–44}. The complex formed between G6PT and G6PC1 (potentially also the complex G6PT/G6PC2) appears to maintain normoglycemia. In contrast, our data show that G6PC3 is needed to maintain neutrophil viability and suggest an important role for glucose in the homeostasis of human neutrophils.

Cheung et al. recently described the phenotype of *g6pc3*-deficient mice that were generated by gene-targeting⁴⁵. These mice had neutropenia and neutrophil dysfunction; another group had previously shown that murine *g6pc3* deficiency results in lowered plasma cholesterol and elevated glucagon levels⁴⁶. We could not identify any consistent aberration in neutrophil function nor any metabolic aberrations in G6PC3-deficient patients. The underlying mechanism of increased apoptosis of neutrophils in the absence of G6PC3 involves increased endoplasmic reticulum stress, usually seen in case of deficient protein folding in the endoplasmic reticulum. In an attempt to counteract potentially toxic effects that may ensue, cells initiate a rescue program which leads, if ineffective, ultimately to apoptosis⁴⁷. Furthermore, we provide evidence that GSK3 β , a key enzyme regulating cellular differentiation and apoptosis³², is implicated in this pathway. In the absence of intracellular glucose, GSK3 β is activated and thus can phosphorylate the anti-apoptotic molecule Mcl1, thereby mediating its degradation^{30, 31}. Neutrophils from G6PC3-deficient patients contain a relative abundance of non-phosphorylated GSK3 β and increased phosphorylation of Mcl1, suggesting that a decrease in antiapoptotic Mcl1 accounts for increased apoptosis in G6PC3-deficient neutrophils. These alterations may at least in part be responsible for the phenotype of G6PC3 deficiency.

While we cannot rule out that additional mechanisms may contribute to the increased level of apoptosis in G6PC3-deficient neutrophils, our data suggest that G6PC3 acts via a pathway involving GSK3 β to maintain viability of neutrophils. Evidence of increased endoplasmic reticulum stress has previously been reported in patients with mutations in *ELA2*^{17, 29}, and premature apoptosis of neutrophils is known to cause the phenotype of congenital neutropenia^{12, 16, 17}. Thus, G6PC3 deficiency provides another example of how increased apoptosis of neutrophil granulocytes causes congenital neutropenia.

Five arguments support our claim that G6PC3-deficiency causes neutropenia: 1) distinct biallelic *G6PC3* mutations were found in two pedigrees and seven singleton patients with congenital neutropenia; 2) sequence analysis predicted that all four missense mutations are likely to affect the function of G6PC3; 3) heterologous expression of G6PC3^{wildtype} and G6PC3^{R253H} in yeast demonstrated that the R253H mutation abrogates enzymatic activity; 4) *g6pc3*^{−/−} knockout mice show a similar phenotype characterized by neutropenia and increased myeloid cell apoptosis and 5) the susceptibility to apoptosis in G6PC3-deficient myeloid cells could be reduced upon retroviral transfer of the wildtype *G6PC3* gene.

Our findings increase the scope of genetic types of congenital neutropenia. and demonstrate a novel role of glucose and/or glucose metabolism in the homeostasis of neutrophil granulocytes..

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Clinical and hematological phenotype

Panel A shows severe paucity of mature neutrophils in bone marrow smears from patient P1. In contrast, bone marrow smears from a healthy individual (Panel B) show normal myeloid differentiation. Panel C illustrates the phenotype of unusual visibility/angiectasia of subcutaneous veins, which was found in all patients from the two pedigrees SCN-I and SCN-II. Panel D shows an atrial septal defect, type II, in patient P3.

SCN-II

Figure 3. Increased susceptibility to apoptosis in myeloid cells and fibroblast cell lines

Panel A shows a FACS plot indicating that peripheral blood neutrophils from patient P5 had increased spontaneous apoptosis as compared to a healthy sibling or a rh-G-CSF treated healthy control (HD) (x axis, Annexin-V; y axis, propidium iodide; logarithmic scales in both axes). Similarly, TNF-alpha induced apoptosis was more pronounced in patients.

Panel B shows an analysis of enhanced apoptosis in patient fibroblasts after exposure to dithiothreitol (DTT) as compared to normal donors.

Panel C shows a FACS plot of *in vitro* differentiated myeloid progenitor cells transduced with a control vector (upper row) or a retroviral vector encoding wildtype G6PC3 (lower row). Cells were treated with tunicamycin and apoptosis was assessed using Annexin-V (x axis) and propidium iodide staining (y axis). G6PC3-transduced patient cells from patient P2 show a decreased susceptibility to apoptosis 16 hours after induction with tunicamycin, as compared to control vector-transduced cells. Data are representative of two independent experiments.

Figure 4. Pathophysiologic consequences of G6PC3 deficiency

Panel A shows the aberrantly enlarged rough ER in myeloid progenitor cells as compared to a healthy individual (Panel B). Panel C indicates increased ER stress in G6PC3-deficient patients, as documented by increased expression levels of Bip mRNA in purified promyelocytes. Panel D represents a Western Blot revealing a decrease in the enzymatically active, dephosphorylated form of GSK3 β in patient P5 after induction with tunicamycin. Panel E shows increased phosphorylation of Mcl1 and increased levels of Bip in neutrophils from a G6PC3-deficient patient (P5) at different timepoints after induction with tunicamycin. Panel F shows 2-deoxyglucose (2DG)-induced dephosphorylation of GSK3 β in 2 healthy donors.

Panel G indicates that healthy neutrophils, but not CD3-positive T cells, are susceptible to apoptosis upon pharmacological glucose-depletion.

Table 1

Clinical and molecular findings in G6PC3-deficient patients

Individual	Sex	Ethnicity/Country of origin	ANC (/μl)*	Infections	Other findings	genotype	Therapy	Current status, height(H) and weight (W) percentiles
1 (P1)	M	Aramean/Turkey	60–246	Neonatal sepsis, otitis media	ASDII, cryptorchidism increased venous marking, intermittent thrombocytopenia, hepatosplenomegaly.	c. [758G>A]+[758G>A] p. [Arg253His]+[Arg253His]	G-CSF, cardiac surgery	6 y, alive H25–50, W3–10
2 (P2)	F	Aramean/Turkey	54–240	Neonatal pneumonia and sepsis	Cor triatriatum, malformation of pulmonary veins, increased venous marking hepatosplenomegaly	c. [758G>A]+[758G>A] p. [Arg253His]+[Arg253His]	G-CSF, cardiac surgery	3y, alive H25, W10–25
3 (P3)	F	Aramean/Turkey	0–61	Pneumonia, sepsis	ASDII, mitral insufficiency increased venous marking, intermittent thrombocytopenia, hepatosplenomegaly	c. [758G>A]+[758G>A] p. [Arg253His]+[Arg253His]	G-CSF, awaiting cardiac correction	11 y, alive H10–25, W10–25
4 (P4)	M	Aramean/Turkey	0–322	Neonatal sepsis	ASDII, cryptorchidism, increased venous marking	c. [758G>A]+[758G>A] p. [Arg253His]+[Arg253His]	G-CSF, cardiac surgery	6y, alive H: 8cm below third percentile W: 1.3kg below third percentile
5 (P5)	M	Aramean/Turkey	25–84	Neonatal sepsis	Increased venous marking, intermittent thrombocytopenia	c. [758G>A]+[758G>A] p. [Arg253His]+[Arg253His]	G-CSF	4y, alive H3, W25
6	F	Turkish/Turkey	90–612	Pneumonia, sepsis	ASDII, pulmonary valve stenosis, paniculitis, increased venous marking.	c. [554T>C]+[554T>C] p. [Leu185Pro]+[Leu185Pro]	G-CSF, awaiting cardiac correction	12y, alive H50, W75
7	F	Caucasian/Greece	30–1280	Perianal abscesses recurrent urinary tract infections	Inner ear hearing loss, increased venous marking	c. [141C>G]+[141C>G] p. [Tyr47X]+[Tyr47X]	G-CSF	13y, alive H25–50, W50–75
8	F	Caucasian/Germany	75–210	Omphalitis recurrent urinary tract infections oral ulcers	ASDII, tracheal fistula, microcephaly, increased venous marking, intermittent thrombocytopenia	c. [784G>C]+[784G>C] p. [Gly262Arg]+[Gly262Arg]	G-CSF, cardiac surgery	7y, alive H3–10, W10
9	F	Caucasian/France	200–500	Upper and lower respiratory tract infections	Myopathy, increased venous marking	c. [677+1 G>A]+[829C>T] p. [?]+[Gln277X]	G-CSF	13 y, alive H3–10, W10
10	M	Caucasian/Germany	0–3000	Urinary tract infection	ASDII, cryptorchidism, genital dysplasia, microcephaly, inner ear hearing loss, increased venous marking, intermittent thrombocytopenia	c. [778G>C]+[778G>C] p. [Gly260Arg]+[Gly260Arg]	G-CSF, cardiac surgery	17 y, alive H: 4 cm below third percentile W: 8kg, below third percentile
11	M	Persian/Iran	250–440	Neonatal sepsis, urinary tract infection	ASDII, patent ductus arteriosus	c. [935dupT]+[935dupT] p. [Asn313fs]+[Asn313fs]	G-CSF, awaiting cardiac correction	10 y, alive H10–25, W10–25
12	M	Arabian/Lebanese	615–2000	respiratory tract infections	Cryptorchidism, bilateral inguinal hernia, cleft palate	c. [144C>A]+[144C>A] p. [Tyr48X]+[Tyr48X]	G-CSF	5 y, alive H3–10, W3–10

* before start of G-CSF,

** during infectious episodes, an occasional increase in neutrophil counts was measured ASDII, atrial septal defect, type II