

Letter to the Editor

Neonatal-onset $T^+B^-NK^+$ severe combined immunodeficiency and neutropenia caused by mutated phosphoglucomutase 3

To the Editor:

Phosphoglucomutase 3 (PGM3) defects were recently identified as a cause of hyper-IgE syndrome (HIES).^{1,2} HIES is classically characterized by recurrent staphylococcal skin abscesses, sinopulmonary infections, and severe eczema with increased plasma IgE concentrations.³ The HIES phenotype can be caused by mutations within a growing list of genes: signal transducer and activator of transcription 3 (*STAT3*), dedicator of cytokinesis 8 (*DOCK8*), tyrosine kinase 2 (*TYK2*), and *PGM3*.

PGM3 catalyzes a key step in uridine diphosphate N-acetylglucosamine synthesis ubiquitously used in glycosylation.⁴ PGM3 was attributed no role in immunodeficiency until 2 groups independently described a homogeneous presentation of HIES in 17 young adults carrying 6 different *PGM3* mutations demonstrated to decrease enzymatic function.^{1,2} The reports made a compelling argument that defective PGM3 causes HIES. Shortly afterward, Stray-Pedersen et al⁵ described 3 unrelated infants also carrying *PGM3* mutations and presenting with homogeneous immunodeficiency. This immunodeficiency debuted soon after birth as T^+B^- natural killer (NK)-positive severe combined immunodeficiency (SCID) together with neutropenia.

To increase knowledge about PGM3 and immunodeficiency, we report the clinical course and detailed laboratory investigations of yet another infant carrying mutated *PGM3*.

The boy was born at term in Denmark as the first child of Syrian-Kurdish parents without known family history of consanguinity, immunodeficiency, or stillbirth. The boy was marginally small for gestational age (birth weight, 3105 g, 5th-10th percentile; length 47 cm, 25th percentile) with dysmorphic facial features: downturned corners of the mouth (Fig 1, A and B), midface hypoplasia (Fig 1, B), micrognathia (Fig 1, B), and natal teeth (Fig 1, C). Limbs were short (Fig 1, D). No eczematous lesions or other atopic features were observed. Investigation revealed horseshoe kidney, atrial septal defect, intestinal malrotation, and bilateral exaggerated trochanter minor (Fig 1, E). Magnetic resonance imaging showed no microcephaly or intracerebral malformations.

At 3 days of age, the boy had respiratory distress and clinical and biochemical signs of sepsis. Intravenous antibiotics ensured rapid recovery, but neutropenia and lymphopenia persisted. Concentrations of monocytes, thrombocytes, and eosinophils were normal. Repeated plasma IgA levels were low, and plasma IgM levels were normal. IgE levels were less than the quantification limit (2 IU/mL). The IgG nadir was 0.6 g/L on day 36. The patient experienced repeated localized and disseminated infectious episodes despite isolation, IgG substitution

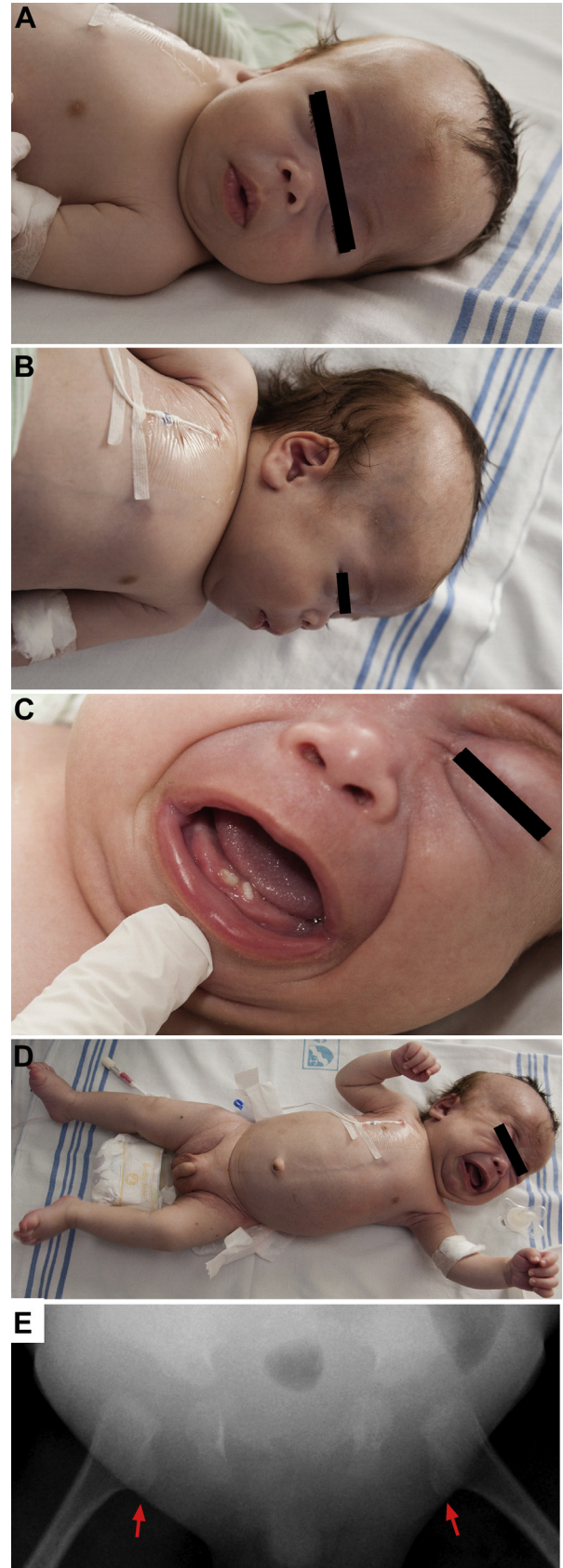


FIG 1. A-D, Photographs of the boy (at age 2 months) showing downturned corners of the mouth (Fig 1, A and B), midface hypoplasia (Fig 1, B), micrognathia (Fig 1, B), natal teeth (Fig 1, C) and short limbs (Fig 1, D). E, X-ray of the hip joints showing bilateral exaggerated trochanter minor (red arrows). Courtesy of consultants Karin Kastberg Petersen and Arne Hørlyck, Department of Radiology, Aarhus University Hospital, Aarhus, Denmark.

TABLE I. Laboratory findings

	Postnatal week				Reference
	3	4	9*	13	
Leukocytes (/μL of blood)					
Neutrophilocytes	240	350	310	340	1200-9600†
Lymphocytes, total	310	900	59	180	1200-8800†
T cells	78	130	19	43	2300-5700
B cells	45	240	22	80	330-2200
NK cells	170	490	15	50	150-920
Of T cells (%)					
CD4 ⁺	44	42	33	42	54-80
CD8 ⁺	20	20	24	22	17-40
CD4 ⁺ CD8 ⁻	34	37	44	35	2.3-6.5
CD4 ⁺ CD8 ⁺	0.90	0.70	0.20	0.50	0.10-1.7
TCR-αβ ⁺	56	49	48	50	92-97
TCR-γδ ⁺	ND	29	48	ND	<10
Of CD4 ⁺ T cells (%)					
Naive (CD45RA ⁺ CCR7 ⁺ CD27 ⁺)	50	53	68	29	80-94
Antigen experienced, total	41	41	25	67	5.7-20
Undefined	9.0	5.7	7.2	4.2	0.18-1.7
Activated (HLA-DR ⁺ and/or CD38 ⁺)	ND	40	ND	35	<5
Of CD8 ⁺ T cells (%)					
Naive (CD45RA ⁺ CCR7 ⁺ CD27 ⁺)	51	35	42	33	37-95
Antigen experienced, total	46	62	55	65	4.8-62
Undefined	3.7	2.6	3.5	2.7	0.0-0.57
Activated (HLA-DR ⁺ and/or CD38 ⁺)	ND	20	ND	25	<5
Of B cells (CD19 ⁺ [%])					
Transitional (IgD ⁺ IgM ⁺ CD38 ⁺ CD27 ⁻)	74	76	27	73	1.6-32
Naive (IgD ⁺ IgM ⁺ CD38 ⁻ CD27 ⁻)	7.1	2.7	63	21	60-92
Switched memory (IgD ⁻ IgM ⁻ CD38 ⁻ CD27 ⁺)	0.043	0.018	0.17	0.23	0.25-1.6
Plasmablast (IgD ⁻ IgM ⁻ CD38 ⁺ CD27 ⁺)	0.30	0.11	0.28	0.46	0.045-1.8
MZ-like (IgD ⁺ IgM ⁺ CD38 ⁻ CD27 ⁺)	0.34	0.048	0.56	0.66	0.50-4.6
Others	18	21	9.0	4.7	1.9-5.1
TRECs (/μL blood)	4	9.4	1.6	ND	39-630‡
KRECs (/μL blood)	55	32	17	ND	8.2-2500‡
Lymphocyte proliferation (% of 2 controls)					
PHA	ND	38	ND	3.0	≥50
PWM	ND	79	ND	29	≥50
Allogeneic cells	ND	15	ND	31	≥50

T cells were categorized as naive, antigen experienced, or undefined according to consensus guidelines.⁶ T-cell receptor and κ receptor excision circles were quantified by mean of real-time quantitative PCR, as described by Sottini et al.⁷ Lymphocyte proliferations were assayed with set numbers of mononuclear cells by means of radioactive thymidine incorporation.

KRECs, κ Receptor excision circles; MZ, marginal zone; ND, not determined; TCR, T-cell receptor; TRECs, T-cell receptor excision circles.

*Ongoing enteropathy.

Reference intervals are central 95 percentiles.

†Local department of biochemistry. Remaining reference intervals were calculated from data of 0-year-olds (n ≥ 10) by means of linear interpolation between closest ranks.

‡Persons were referred for blood typing. Remaining persons were referred for immunodeficiency testing (SCID and lymphopenia ruled out).

(initiated day 37), and prophylactic antimicrobial chemotherapy (fluconazole, acyclovir, co-trimoxazole, and amoxicillin). Pathogens could only occasionally be verified (all commensal skin staphylococci: *Staphylococcus epidermidis* and coagulase-negative species). Anemia developed in the second postnatal week, necessitating red cell transfusion. Bone marrow examination uncovered low cell density, but results were otherwise normal. The abdomen distended on day 33 as a first sign of progressing protein-losing enteropathy, necessitating daily albumin substitution, higher IgG substitution dosage (maximum, 600 mg/kg/wk), and total parenteral nutrition. Repeated microbiological stool evaluations were normal. Endoscopic biopsy specimens (conventional and electron microscopy) were normal. The enteropathy resolved on supporting care.

Further laboratory investigations of peripheral blood (Table I)^{6,7} included flow cytometry, which initially showed the

T⁻B⁻NK⁺ lymphocyte phenotype but later also decreased NK cell concentration. Lymphocyte proliferation test results were moderately low, and high percentages of T cells were activated, antigen experienced, double-negative (CD4⁻CD8⁻), and T-cell receptor γδ expressing, leading us to suspect hypomorphic mutations in recombination-activating gene 1 (*RAG1*) or *RAG2*, but results of Sanger sequencing were normal. Therefore whole-exome sequencing of the boy and his parents was initiated. Despite the overwhelming infectious burden, low percentages of B cells had undergone peripheral differentiation (eg, to plasmablasts) based on their phenotypes. Most B cells were transitional, but up to 21% were unclassifiable (possibly immature: CD19⁺CD21⁺IgD⁻IgM⁺CD27⁻CD38⁺). T-cell receptor excision circle concentrations were low in accordance with low naive T-cell concentrations. In contrast, κ receptor excision circle concentrations were normal, implying a high κ receptor excision

circle concentration per B cell in accordance with their sparse peripheral differentiation. *TCRγ* gene rearrangements were oligoclonal, whereas *IgH* gene rearrangements were not abnormal (PCR and fragment length analysis, data not shown). We find the results support defective central T-cell development, whereas peripheral development can be more affected within the B-cell lineage.

At 3 months of age, the boy was transferred to the Danish National Center for Hematopoietic Stem-cell Transplantation in Children without a definitive diagnosis. The enteropathy reappeared, but again, no cause could be established. He died 5 months later from multiorgan failure caused by chemotherapy conditioning (treosulfan, fludarabine, and thymoglobulin) before transplantation.

Whole-exome sequencing data were analyzed by using Ingenuity Variant Analysis software (Ingenuity, Redwood City, Calif) to identify rare recessive mutations possibly related to SCID and of predicted functional importance (using data tools SIFT⁸ and Polyphen2⁹). Homozygous, rare, and potentially disease-related sequence variants were identified in 4 different genes, *PGM3*, *GTF2E1*, *KHDRBS2*, and *KRT20*, none of which were known to be associated with SCID at the time (June 2014). Some parental relatedness was strongly suggested by large stretches of homozygosity in the boy's exome (up to 45 MB per chromosome).

Shortly afterward, Stray-Pedersen et al⁵ reported 3 unrelated infants carrying *PGM3* mutations and presenting with a very similar SCID phenotype. One of these, an Afghan girl, carried the exact same *PGM3* variant as the boy reported: *PGM3*, NM_015599.2, c.[737A>G];[737A>G], p.(Asn246Ser). Strikingly, the 2 infants shared various dysmorphic features, including downturned corners of the mouth, midface hypoplasia, micrognathia, short limbs, and bilateral exaggerated trochanter minor, and both were anemic. The boy's additional malformations may be linked to other homozygotic parts of his genome. We Sanger sequenced *PGM3*, confirming that the boy was homozygous for the variant and that the parents were heterozygote carriers.

The 2 independent reports of homozygous *PGM3* N246S in 2 infants from distinct world populations presenting very similar clinical and laboratory findings strongly suggest that this variant causes T⁺B⁺NK⁺ SCID with neutropenia. The variant is not present among the 162 missense *PGM3* variants in the ExAC database, which contains data of 60,706 subjects (<http://exac.broadinstitute.org> [accessed April, 2015]), suggesting a rare carrier status rather than a hot spot for *de novo* mutations.

It is of interest to learn why some *PGM3* mutations cause SCID and others cause HIES. Residual PGM3 function is likely

important. We did not test the enzymatic function related to N246S, but Stray-Pedersen et al⁵ reported that this substitution effectively abolished PGM3 function (residual function, approximately 1%). The other SCID-related *PGM3* mutations were hypomorphic causing considerable residual function (approximately 50%),⁵ whereas the HIES-related *PGM3* mutations actually caused lower residual function (approximately 20% to 30%).^{1,2} We speculate that the *in vivo* effects of hypomorphic *PGM3* mutations are modified by variants in other genes, thereby determining whether SCID or HIES develops. Future studies must clarify this to enable counseling and appropriate treatment of persons carrying hypomorphic *PGM3* mutations.

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