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LETTER TO EDITOR

Compound Heterozygous *PGM3* Mutations in a Thai Patient with a Specific Antibody Deficiency Requiring Monthly IVIG Infusions

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To the Editor:

Phosphoglucomutase 3 (PGM3) is an enzyme converting *N*-acetyl-glucosamine-6-phosphate to *N*-acetylglucosamine-1-phosphate, a sugar nucleotide critical for glycosylation pathways. PGM3 defects have been reported in 41 patients of 16 families with immunodeficiency [1–8]. Mutations in *PGM3* were first reported in 17 patients with hyper-IgE syndrome (HIES) [1, 2]. Subsequently, patients with *PGM3* mutations were found to have varying degrees of immunological abnormalities including T-B-severe combined immunodeficiency [4–6], diminished T cell function with high immunoglobulins including IgE [7, 8], and normal IgE with mild immunodeficiency [3]. Herein, we report the clinical course and detailed laboratory investigations of a Thai patient carrying novel mutations in *PGM3*.

The boy was born at term to non-consanguineous Thai parents. He was the second child, and his older brother was healthy. His parents were both healthy with no histories of immunodeficiency or infantile death in their families. The patient presented with severe atopic dermatitis at the age of 2 months. During infancy, he had chronic diarrhea, recurrent otitis media, recurrent pneumonia, salmonella and candida septicemia, a severe varicella infection, and multiple food allergies. At the age of 2 years, he was referred to our hospital for further immunological evaluation. Investigations revealed increased IgE (13,877 IU/mL; normal range 2–97 IU/mL) and IgG (1320 mg/dL; normal range 453–916 mg/dL) but normal IgA (30 mg/dL; normal range 20–100 mg/dL) and IgM (50 mg/dL; normal range 19–146 mg/dL) levels. Lymphocyte phenotype showed low CD3 (730 cells/μL; normal range 2100–6200 cells/μL), CD4 (300 cells/μL; normal range 1300–3400 cells/μL), CD8 (430 cells/μL; normal range 620–2000 cells/μL), and CD4:CD8 ratio (0.69), with very low CD 19/20 (30 cells/μL; normal range 720–2600 cells/μL). PHA lymphocyte proliferation testing was within the normal range. He had protective tetanus antibody titers but inadequate responses to the 23-valent pneumococcal polysaccharide vaccine. The patient underwent molecular testing using Sanger sequencing of *STAT3, DOCK8*, and *TYK2*, which showed no pathogenic variants. Therefore, exome sequencing (ES) of the boy and his parents was performed. The Agilent SureSelect Human All Exon kits version 4 were used for exon capturing, exome enrichment, and library preparation followed by sequencing of the post-captured libraries on the Illumina HiSeq 4000 Sequencer. Filtering criteria for candidate variants include the allele frequencies of less than 1% in our 2166 in-house Thai exomes, The Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org) and 1000G databases. Prediction software including SIFT, Polyphen, and MCAP was used [9].

Trio ES revealed that the patient was compound heterozygous for the novel c.1003A>G (p.Thr335Ala) missense in exon 8 and c.1443delC (p.Asn482Metfs*4) frameshift mutations in exon 12 (NM_015599.2). Both mutations were

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confirmed by PCR and Sanger sequencing (Fig. 1a); they are inherited from the mother and the father, respectively. The maternal c.1003A>G missense variant is found to affect an evolutionarily highly conserved amino acid residue 335 (Fig. 1b). This mutation was located near the sugar-binding domain of PGM3 and predicted to be deleterious by SIFT, damaging by PolyPhen-2 HVAR, and possibly pathogenic by MCAP algorithms. The paternal 1-bp deletion (c.1443delC) results in a frameshift leading to a premature stop codon. The unaffected brother was shown to be heterozygous for the paternal c.1443delC mutation but did not harbor the maternal missense (Fig. 1a).

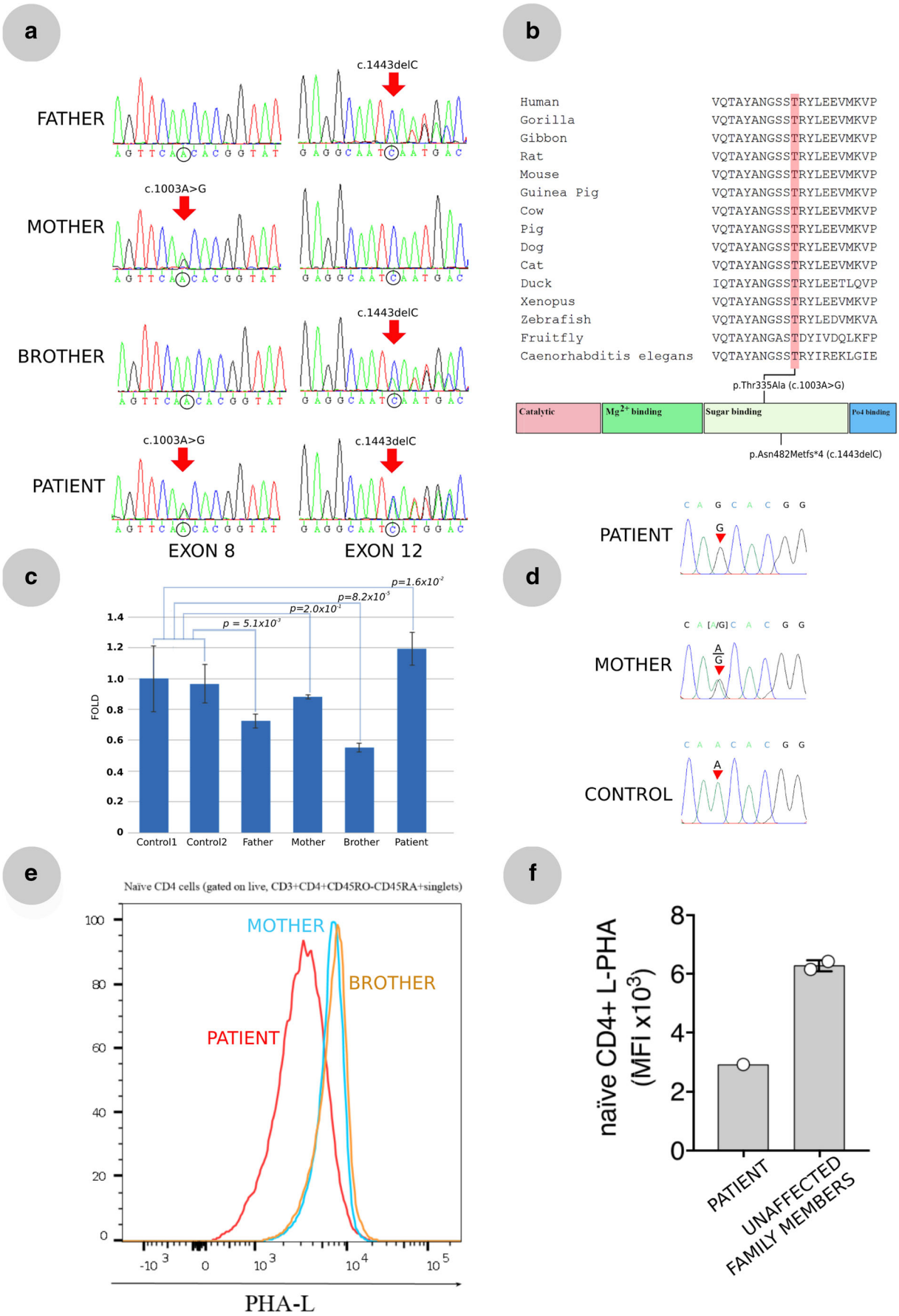
To determine the expression level of *PGM3*, total RNA was extracted from the leukocytes of the patient, his unaffected brother, his parents, and two age- and sex-matched controls. Real-time PCR was performed using the hs00985101_m1 TaqMan kit from Thermo Scientific, Inc. (MA, USA). The probe sequence was 5′-ACAGGCTTCTTGCAGTGGAG-3′ spanning junctions of exons 5 and 6 of *PGM3*. To normalize the *PGM3* expression levels, *ACTB* was used as an internal control using the hs02458991_g1 TaqMan kit from Thermo Scientific, Inc. We found that *PGM3* expressions in the father and the brother were significantly lower than those of the controls, while that of the index patient was significantly increased relative to the controls (Fig. 1c; Supplementary Table 1).

Subsequently, we conducted a reverse transcription using total RNA from leukocytes, followed by PCR using a forward primer spanning exons 3 and 4, 5′-TAGAGATACCAGGC CCAGCA-3′, and a reverse primer spanning exons 12 and 13, 5′-TGCACTTTCTTGTGAGTCTGC-3′. The PCR products were subjected to Sanger sequencing using an internal primer spanning exons 6 and 7, 5′-TCATCAGAAACCTC CACAGG-3′. While the mother showed equal expression of the wild-type and the missense c.1003A>G alleles, the patient showed only the c.1003A>G allele (Fig. 1d). This suggests that the paternal c.1443delC-mutated RNA is destroyed probably by nonsense-mediated mRNA decay (NMD). We hypothesize that insufficient PGM3 functions in patient cells result in a feedback mechanism that increases its transcription, as shown in Fig. 1c. Alternatively, significant abnormalities in the cellular composition of PBMCs—including naive lymphocyte cytopenias—relative to healthy age-matched volunteers and his unaffected family members we have identified in the index patient may have impacted the total PGM3 transcript levels detected. However, protein expression analysis was not done in this study, and thus, absent functions of the c.1443delC has not been confirmed. In order to demonstrate that the expressed p.Thr335Ala mutant PGM3 enzyme had reduced functional activity, ex vivo PBMCs were stained with Live/Dead Fixable Blue viability dye (Invitrogen, Carlsbad, CA), anti-CD3 ACP-H7 (BioLegend, San Diego, CA), anti-CD4 BUV395, anti-CD45RA BV711 (BD Biosciences, San Jose, CA), and anti-CD45RO TRPE (Beckman-Coulter, Pasadena, CA) as well as fluorescein-conjugated L-PHA (Vector Laboratories, Burlingame, CA) as described, in order to quantify *N*-glycan complexity on naive CD4+ cells which has been shown to be dependent upon intact PGM3 activity and significantly reduced in patients with hypomorphic recessive *PGM3* mutations [10]. The patient's PHA fluorescent intensity was approximately half that of his unaffected mother and brother, consistent with impaired enzymatic activity of PGM3 (Fig. 1e, f).

The child has been treated with cotrimoxazole prophylaxis since the age of 2 years and regular intravenous immunoglobulin (IVIG) every 4 weeks since the age of 5 years. Currently, at the age of 9 years, his food allergies have persisted as has his mild atopic dermatitis and developmental delay in gross motor, fine motor, social, and language skills. His developmental age is equivalent to a 5-year-old boy. However, he has had no additional severe infections requiring hospitalization.

Hypomorphic *PGM3* mutations lead to aberrant glycosylation likely affecting a large number of immunologically relevant glycoproteins. The degree of immunodeficiency in patients with *PGM3* mutations ranges from being mild with normal or high IgE [1–3, 8] to severe combined immunodeficiency [4–6]. The differences in the severity of the clinical manifestations of patients with *PGM3* mutations can possibly be explained by the degree of residual PGM3 enzyme activity and resulting alterations in protein glycosylation within cells. A lesser degree of PGM3 enzymatic activity has been reported to correlate with severe combined immunodeficiency phenotype [4], but genotype-phenotype associations may not be clear-cut in this disorder. In addition to important immunological impairments, patients with *PGM3* mutations also commonly have dysmorphic features [4–6], skeletal abnormalities [1, 2, 4–6], and varying neurological abnormalities [1, 2, 4, 7]. Variable effects on immune cell numbers have also been

Fig. 1 **a** Electropherograms show the genomic DNA sequences of the maternal *PGM3* c.1003A>G missense (left panel) and paternal c.1443delC (right panel) variants in the patient, and unaffected family members. **b** The novel p.Thr335Ala variant affecting the amino acid residue 335 which is evolutionarily conserved from humans to *Caenorhabditis elegan*. Schematic of PGM3 domains shows N-terminal catalytic (pink), Mg^{2+}-binding (green), central sugar-binding (light green), and C-terminal PO4 binding (blue) loops, respectively. **c** RNA expression levels of *PGM3* relative to *ACTB*. Relative mean RNA expression levels of *PGM3* in total leukocyte from the patient with PGM3 mutations, unaffected family members (father, mother, and brother), and two control samples. Combined data from three independent experiments were analyzed using one-way analysis of variance (ANOVA). **d** Electropherograms of the patient, his mother, and a control showing that the patient had only *PGM3* RNA from the c.1003A>G allele. Histograms (**e**) and quantification (**f**) of PHA-L geometric mean fluorescent staining intensity measured in naive $CD4^+$ T cells from the PGM3-deficient patient (red), as well as his unaffected mother (blue) and unaffected brother (orange)


a
FATHER
MOTHER
BROTHER
PATIENT
c.1443delC
c.1003A>G
EXON 8
EXON 12
b
Human VQTAYANGSSTRYLEEVMKVP
Gorilla VQTAYANGSSTRYLEEVMKVP
Gibbon VQTAYANGSSTRYLEEVMKVP
Rat VQTAYANGSSTRYLEEVMKVP
Mouse VQTAYANGSSTRYLEEVMKVP
Guinea Pig VQTAYANGSSTRYLEEVMKVP
Cow VQTAYANGSSTRYLEEVMKVP
Pig VQTAYANGSSTRYLEEVMKVP
Dog VQTAYANGSSTRYLEEVMKVP
Cat VQTAYANGSSTRYLEEVMKVP
Duck IQTAYANGSSTRYLEETLQVP
Xenopus VQTAYANGSSTRYLEEVMKVP
Zebrafish VQTAYANGSSTRYLEDVMKVA
Fruitfly VQTAYANGASTDYIVDQLKFP
Caenorhabditis elegans VQTAYANGSSTRYIREKLGIE
p.Thr335Ala (c.1003A>G)
Catalytic
Mg2+ binding
Sugar binding
Po4 binding
p.Asn482Metfs*4 (c.1443delC)
c
p=1.6x10-2
p=8.2x10-5
p=2.0x10-4
p = 5.1x10-3
FOLD
Control1 Control2 Father Mother Brother Patient
d
PATIENT
MOTHER
CONTROL
e
Naïve CD4 cells (gated on live, CD3+CD4+CD45RO-CD45RA+singlets)
MOTHER
BROTHER
PATIENT
PHA-L
f
naïve CD4+ L-PHA (MFI x10³)
PATIENT
UNAFFECTED FAMILY MEMBERS

reported, including neutropenia [1, 2, 4–6], lymphopenia [1–8], eosinophilia [1, 3, 7, 8], decreased T cell and B cell numbers [1–8], and decreased NK cell number [2, 3, 6, 7]. The level of serum IgE ranges from normal [3, 5] to highly increased [1, 2, 4, 6–8] with widely variable changes of IgG, IgA, and IgM levels. These immunologic impairments result in a combined immunodeficiency characterized by recurrent bacterial, viral, and fungal infections as well as severe manifestations of clinical allergy in most patients. Our patient presented with recurrent skin and sinopulmonary infections, severe varicella infection, aopic dermatitis, food allergy, and developmental delay. However, he had no dysmorphic features or skeletal dysplasia. Clinical findings and laboratory data of previously reported patients with *PGM3* mutations and our patient are summarized in Table 1. The phenotype of our patient may be due to the greater residual enzymatic activity of the PGM3 enzyme relative to patients with SCID or more pervasive phenotypes such as skeletal dysplasia. A recent study have demonstrated the impaired STAT3 signaling downstream of the highly glycosylated protein gp130. This

Table 1 Summary of published clinical findings and laboratory data for patients with *PGM3* mutations

	The present patient	Sassi et al. [1]	Zhang et al. [2]	Lundin et al. [3]	Stray-Pedersen et al. [4]	Bernth-Jensen et al. [5]	Pacheco-Cuellar et al. [6]	Ben-Khemis et al. [7]	Lundin et al. [8]
Clinical findings									
Recurrent staphylococcal/skin infections	Yes	8/9	6/8	Yes	3/3	Yes	NR	10/12	Yes
Recurrent sinopulmonary infections	Yes	9/9	8/8	Yes	3/3	NR	NR	10/12	Yes
Eczema	Yes	7/9	8/8	Yes	3/3	No	NR	10/12	Yes
Food allergy	Yes	NR	5/8	No	NR	NR	NR	NR	Yes
Asthma/AR	Yes	NR	6/8	NR	NR	NR	NR	NR	NR
Candida infection	Yes	6/9	NR	No	NR	NR	NR	5/12	Yes
Viral infections	Yes	4/9	6/8	Yes	NR	NR	NR	5/12	Yes
Skeletal dysplasia	No	NR	NR	No	2/3	Yes	2/2	NR	No
Scoliosis	No	1/9	4/8	No	NR	NR	No	6/12	No
Dysmorphic facial features	No	NR	NR	No	2/3	Yes	2/2	NR	No
Developmental delay/intellectual disability	Yes	6/7	7/8	No	2/3	NR	NR	9/12	No
Laboratory data									
Peripheral blood count									
Neutrophils (< 1500 cells/μL)	Yes	↓2/7	↓4/8	No	↓3/3	Yes	2/2	NR	No
Lymphocytes (< 1500 cells/μL)	Yes	↓2/7	↓5/8	Yes	↓2/3	Yes	2/2	3/8	Yes
Eosinophils (> 500 cells/μL)	Yes	↑7/7	No	Yes	NR	No	No	5/10	Yes
Lymphocyte subset									
CD3	↓	↓4/7	NR	↓	↓↓3/3	↓↓	↓↓	↓6/8	↓
CD4	↓	↓6/7	↓5/7	↓	NR	↓↓	↓↓	↓↓8/8	↓
CD8	↓	↓7/7	↓5/7	↓	NR	↓↓	↓↓	↓3/8	↓
CD19,20	↓↓	↓4/7 ↑1/7	↓6/7	↓	↓↓3/3	↓↓	↓↓	↓6/8	Normal
CD16/56	Normal	↑4/7	↓2/7	↓	Normal	Normal	↓	↓2/8	Normal
Serum immunoglobulin									
IgG	↑	↑3/7	↑3/7	Normal	↓1/3	↓	NR	NR	↑
IgA	↑	↑5/7	↑5/7	↑	↓1/3	NR	NR	NR	↑
IgM	Normal	↑3/7 ↓1/7	Normal	↓	↓2/3	NR	NR	NR	↑
IgE	↑↑	↑↑7/7	↑↑7/7	Normal	↑1/3	Low	NR	↑↑10/11	↑↑
In vitro T cell PHA stimulation test	Normal	Normal	NR	Normal	NR	Impaired	NR	Impaired 7/7	Impaired
Antibody titer to bacteria									
Tetanus	Normal	NR	Normal	NR	NR	NR	NR	NR	NR
Streptococcus pneumoniae	↓	NR	Normal	NR	NR	NR	NR	NR	NR

NR not reported

may explain some clinical features shared by PGM3 deficiency and STAT3-HIES [11]. Furthermore, our patient is one of only a few patients reported to be from a non-consanguineous union with compound heterozygous mutations [2, 4]; in such individuals, additional single-gene disorders, which have been reported to be present in 4.9% of sequenced individuals [12], are much less likely to be present and provide the clearest evidence for the clinical consequences of focal impairment of PGM3 function.

In conclusion, we report two novel *PGM3* mutations resulting in PGM3 insufficiency and impaired leukocyte glycosylation in a patient with a phenotype that includes food allergy, intellectual disability, recurrent infections, high IgE, and specific antibody deficiency. This 9-year-old patient has not suffered severe infections requiring hospitalization since the initiation of the monthly IVIG and prophylactic antibiotics.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This study was reviewed and approved by the human rights and ethic committee of Faculty of Medicine, Ramathibodi Hospital, Mahidol University (ID 10-61-27).

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