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Novel *PGM3* compound heterozygous variants with IgE-related dermatitis, lymphopenia, without syndromic features

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Abstract

Background: Phosphoglucomutase-3 (*PGM3*) deficiency is a congenital disorder of glycosylation (CDG) with hyperimmunoglobulin IgE, atopy, and a variable immunological phenotype; most reported patients display dysmorphic features.

The aim of the study was to characterize the genotype and phenotype of individuals with newly identified compound heterozygous variants in the phosphate-binding domain of *PGM3* in order to better understand phenotypic differences between these patients and published cases.

Methods: We analyzed *PGM3* protein expression, *PGM3* enzymatic activity, the presence of other gene variants within the N-glycosylation pathway, and the clinical and immunological manifestations of two affected siblings.

Results: Patients belonged to a non-consanguineous family, presenting with atopic dermatitis, elevated levels of IgE, and CD4⁺ lymphopenia (a more severe phenotype was observed in Patient 2), but lacked dysmorphic features or neurocognitive

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impairment. Compound heterozygous *PGM3* variants were identified, located in the phosphate-binding domain of the enzyme. *PGM3* expression was comparable to healthy donors, but L-PHA binding in naïve-CD4+ cells was decreased. Examination of exome sequence identified the presence of one additional candidate variant of unknown significance (VUS) in the N-glycosylation pathway in Patient 2: a variant predicted to have moderate-to-high impact in *ALG12*.

Conclusions: Our analysis revealed that L-PHA binding is reduced in naïve-CD4+ cells, which is consistent with decreased residual *PGM3* enzymatic activity. Other gene variants in the N-glycosylation pathway may modify patient phenotypes in *PGM3* deficiency. This study expands the clinical criteria for when *PGM3* deficiency should be considered among individuals with hyper-IgE.

KEYWORDS

dermatitis, glycosylation deficiency, hyper-IgE syndrome, inborn error of immunity, *PGM3* deficiency

1 | INTRODUCTION

Glycosylation is an important and complex co- and post-translational protein modification system involving at least 1%-2% of all human genes.¹ It is the process by which carbohydrate chains, or glycans, are covalently linked to lipids or proteins. Congenital disorders of glycosylation (CDG) encompass over 130 genetic conditions spanning a broad multisystem clinical spectrum.² The most common manifestations include neurological abnormalities with abnormal magnetic resonance imaging findings,³ skin manifestations, coagulopathy, and immunodeficiency.^{4,5} Eight biochemical pathways are involved in glycosylation, with the N- and O-glycosylation of proteins being the best known. The N-glycosylation of proteins involves¹ the synthesis of a precursor oligosaccharide attached to a dolichophosphate (dol-P) acting as a transporter,² the transfer of this oligosaccharide to the protein in the endoplasmic reticulum (ER), and lastly³ the processing of the oligosaccharide via the elimination and/or addition of different sugars, which, in humans, leads to more complex and specialized glycans in the ER and Golgi apparatus. The O-glycosylation of proteins is rather more diverse and gives rise to highly branched oligosaccharide structures occurring in the Golgi apparatus⁶ (Figure 1). N-glycosylation defects are studied through an inexpensive and often reliable biomarker test, the glycosylated transferrin isoform analysis, leading to a classification of CDG into 2 main groups: type I profile, characterized by defects in the assembly or transfer of the dolichol-linked glycan, and type II, which involves processing defects of the glycan.

Patients with CDG have extremely variable expressivity of associated phenotypes, and severity can vary among individuals with the same subtype, and even among members of the same family. It has been suggested that prevalent polymorphisms in other genes in glycosylation pathways may have a role in the final phenotype^{7,8} but to date, this is an unproven hypothesis.

Key message

Phosphoglucomutase-3 (*PGM3*) deficiency is a congenital disorder of glycosylation included within the hyper-IgE syndromes, for which most reported patients display syndromic features. In this article, we fully characterize the genotype and phenotype of newly identified compound heterozygous variants by means of *PGM3* protein expression and functional activity, exploring the presence of other gene variants within the N-glycosylation pathway that may contribute to the final phenotype of patients. This report expands the warning signs for suspecting *PGM3* deficiency in the allergy clinic in patients with hyper-IgE, eosinophilia, and dermatitis and provides insight into the factors that contribute to the phenotype in *PGM3* deficiency, which can be extended to other congenital disorders of glycosylation and related primary immunodeficiencies, such as *MAGT1* deficiency and *MAN2B2* deficiency.

Glycosylation is pivotal in immunity: Various relevant molecules of both the innate and adaptive immune system are glycosylated, and their correct glycosylation determines cell-to-cell communication as well as immune pathway signaling. This would explain why at least 23 CDGs have been reported to include immune deficiency as a prominent phenotype,^{9,10} listed in Table S1.

There are an increasing number of rare monogenic syndromes characterized by variable infectious susceptibility, atopic skin inflammation, and significant elevations of total serum IgE. The first of these disorders to be well-characterized and genetically defined is the autosomal dominant hyper-IgE syndrome (AD-HIES,

TABLE 1 Summary of immunological and other laboratory data in the siblings with PGM3 deficiency. Comparison with other syndromes with hyper-IgE^{12,30}

Genes involved in HIES	Lymphopenia**	T-cell proliferation (PHA)	Treg	Memory and Switched-m B cells	Specific Ab production	Elevated IgA	Elevated IgE (eosinophilia)	Th17	Transferrin
PGM3	yes ¹	V	N (D in SCID forms)	D	N/D (auto-immunity)	yes	+++ (eosino-phililia)	N or I (D in SCID forms)	N
PGM3-P1	yes ² 1000/mm ³ (1400-3300)	N > 50% (normal > 50)	N 2.1% (1-3.7)	N Memory 21.4% (5.0-60.0) Switched 14.9% (3.0-46.0)	N Pneumo-coccal basal IgG: 644 UI/L (>250)	No 1.3g/L (4.6-2.2)	++ 2383 KU/L (0-230) (500/mm ³ (0-500))	N 10.6% (5-11)	N ⁶ 3.6% (1.6-6.5)
PGM3-P2	yes ³ 500/mm ³ (1400-3300)	N > 50% (normal > 50)	N 4.1% (4-20)	N Memory 13.6% (5.0-60.0) Switched 3.8% (1.0-43.0)	N Pneumo-coccal basal IgG: 305 UI/L (>250)	Yes 4.4g/L (4.6-2.2)	++ 5724 KU/L (0-230) (200/mm ³ (0-500))	N 2.7% (>0.5)	N ⁶ 3.4% (1.6-6.5)
STAT3 dominant negative ³²	no ⁴	N	I	D	D (capsulated)	no	+++ (eosino-phililia)	D	N
IL6R	no	N	?	D	D	no	+++	D	N
IL6ST	no	N	?	D	N/D	no	++ (eosino-phililia)	D	N
ZNF341	no	N	?	D	D	no	++	D	N
LDS***	no	N	I ³³	N	N				N
ERBIN	no	N	I	N	N	no	++	N	N
TGFBR	no	N	N	N	N	no	++	N	N
DOCK8*	yes ⁵	D	D	D	D	yes IgM typically decreased.	+++ (eosino-phililia)	N/D (Th2 skew)	N

Abbreviations: N: normal; V: variable; I: increased; D: decreased; ? include the normal range according to age.

*DOCK8 deficiency currently classified as combined immunodeficiency and not HIES¹²**Lymphopenia: T-cell subpopulations affected: ¹ CD4+ and CD8+ lymphopenia (more pronounced in CD4+); ² P1: CD4+ T-cell lymphopenia (CD4+ 282 NR: 500-2000), normal gamma-delta TcR expression, low naive CD3+ CD4RA+ (28.6%, NR: 56-95) and increased memory CD3+ CD45RO+ (54.7%, NR: 2-35), with normal Th17 cells expression; ³ B, T, and NK lymphocytes are in the lower normal range, slight gamma-delta TcR expansion (17.7%, NR: 0.83-11), low naive CD3+ CD4RA+ (15.8%, NR: 56-95), and increased memory CD3+ CD45RO+ (59%, NR: 2-35), with normal Th17 cells' expression; ⁴ reduced central memory T cells; ⁵ progressive lymphopenia, reduced recent emigrants, CD8+ exhaustion pattern, gamma-delta expansion, NK and NKT cells' reduction; ⁶ Values of trisialotransferrin are expressed in the percentage of area under the curve.

***LDS: Loeys-Dietz syndromes caused by TGFBR1, TGFBR2, or SMAD3 mutations have been described associated with hyper-IgE and atopy.

2 | METHODS

Blood samples were obtained from each patient for the preparation of plasma/serum and nucleic acids, in accordance with the Helsinki Declaration of 1964, as revised in October 2013 in Fortaleza, Brazil. Written informed consent from the patients' parents/guardians was dutifully obtained as required, including on the NIAID IRB-approved protocol (NCT00852943).

Primary human fibroblasts were obtained from biopsy specimens from P1 (Patient 1), P2 (Patient 2), and healthy controls and were cultured in DMEM (Invitrogen) supplemented with 10% FCS. They were then transformed with an SV40 vector, as previously described,²⁶ to create immortalized fibroblast cell lines: SV40 fibroblasts.

Plasma/serum isofocusing of transferrin (Trf) and the quantification of tetra-, bi-, and a-sialo glycoforms (bearing two, one, or zero bi-antennary mature N-glycan chains in CDG-I) was undertaken using capillary zone electrophoresis (CZE) method for P1 (CEofix -CDT kit, Analis, Suarlée, Belgium; P/ACE 5000 system with UV detector, Beckman-Coulter, Fullerton, CA, USA) as previously described.²⁷

To screen the genetic background, we evaluated a selection of previously reported frequent variants on CDG genes as potential modifiers.^{7,8} Some of them are predicted as having mild-to-moderate impact by widely used predictors (PolyPhen2, SIFT, MutationTaster).⁷

Enzymatic activity was functionally evaluated by determining the degree of N-glycan complex branching using lectin-based flow cytometry.²⁸ Briefly, to determine the extracellular expression of complex N-glycans, patient PBMCs were stained with fluorescein-conjugated L-PHA lectin (Vector Laboratories) in live, CD45RO-CD45RA + CD4+T cells, and geometric mean fluorescence was measured using flow cytometry.

2.1 | Human molecular genetics and whole exome sequencing (WES)

WES was performed as indicated in Appendix 1.

2.2 | Immunoblots

Human PBMCs were isolated with Ficoll-Hypaque density gradient centrifugation (Amersham-Pharmacia-Biotech, Buckinghamshire, UK) from whole-blood samples obtained from P1, his relatives and healthy volunteers. Total cell extracts were prepared from PBMCs. Equal amounts of protein from each sample were separated by SDS-PAGE and blotted onto iBlot Gel Transfer Stacks (Invitrogen). These nitrocellulose membranes were then probed with PGM3 Polyclonal Antibody (PA5-56249, Invitrogen, Carlsbad, CA, USA), followed by a secondary anti-rabbit IgG-HRP linked antibody (Cell Signaling, Beverly, MA, USA). Membranes were stripped and reprobed with an antibody against GADPH (Abcam), to control for protein loading. Antibody binding was detected with enhanced chemiluminescence (ECL; Amersham-Pharmacia-Biotech).

3 | RESULTS

3.1 | Clinical report

We investigated two siblings aged 23 (P1) and 11 (P2) years old, both girls, from a non-consanguineous family of Caucasian descent. Clinical features are summarized in Table S3. Both presented with serum IgE levels ranging between 4,652-23,969 KUI/L, significant CD4⁺ lymphopenia, and normal IgG and IgM levels; P2 had elevated IgA levels, as has been reported.^{15,17} Transferrin levels and sialotransferrin were normal in both patients, as were coagulation tests. Although P2 had increased liver echogenicity, neither of them had signs of liver impairment. Evaluation by a clinical geneticist only identified micrognathia in P2 (Figure S1), without neurological symptoms, except for an attention deficit disorder and hyperactivity in P2 ascribed to severe dermatitis and pruritus; both subjects were of normal intelligence. Complete immune workup is summarized in Table 1, as well as cytokine profile in Figure 3B.

3.2 | Compound heterozygous variants in PGM3 in two siblings with severe IgE-related dermatitis and T-cell lymphopenia

WES was performed for both patients with the Agilent SureSelect Human All Exon 51Mb kit. Next-generation deep sequencing identified 777,086 and 779,718 coding variants, after alignment and variant calling. Because patients were from a non-consanguineous family, analyses were performed for all the variants, retaining only the variants not reported in the NCBI dbSNP 134 and 1000 Genomes (<http://www.1000genomes.org/>) databases. We excluded synonymous variants and checked the quality of the exome sequences; we then identified compound heterozygous variants in PGM3 exon 12 (g.83120412 G > A and g.83120453 G > A). At the protein level, these variants yielded the missense variants, p.T464I and a premature stop codon p.Q478X. Neither variant has previously been reported in available databases. These variants segregated with disease in the pedigree (both sisters carried the compound heterozygous variants, each parent carried one of the variants) and were validated by Sanger sequencing of gDNA from peripheral leukocytes (Figure 2A). No other variants were found in the coding region of PGM3 nor in other genes related to IEI. These data suggested that compound heterozygous variants (g.83120412 G > A and g.83120453 G > A) in PGM3 may be responsible for the novel autosomal recessive form of IEI in P1 and P2.

3.3 | Expression and function of PGM3 in cells from the patients

PGM3 protein expression levels in PBMCs and SV40 fibroblasts from the affected siblings were comparable to those of healthy donors on immunoblotting (Figure 2B). Protein modeling predicts

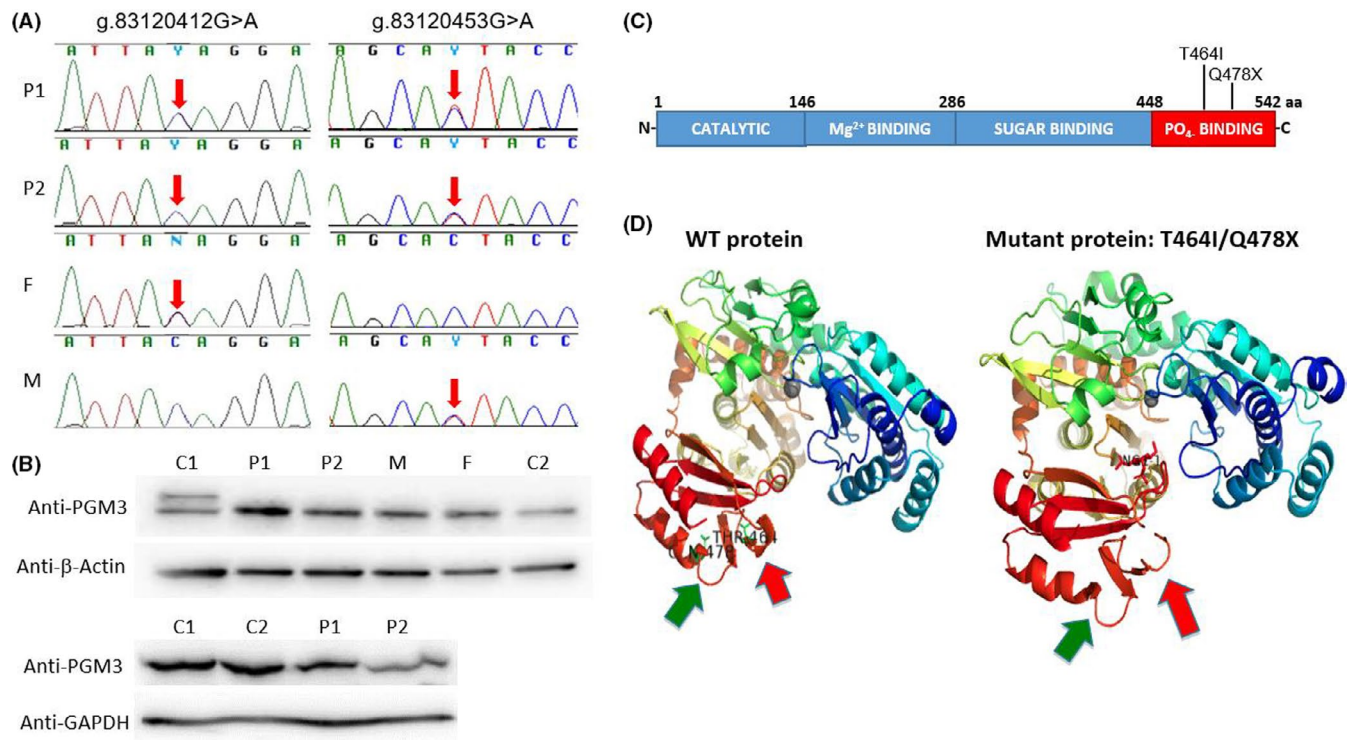


FIGURE 2 Compound heterozygous *PGM3* variants in siblings with IgE-related dermatitis and T-cell lymphopenia. A, Electropherograms showing the Sanger sequencing results of the variant site in *PGM3* from gDNA extracted from leukocytes of patients and parents. The variants g. 83120412 G > A and g. 83120453 G > A, compound heterozygous in patients and heterozygous in parents are indicated with red arrow. P1 and P2 are patients, F is the father, and M is the mother. B, Immunoblot analysis of *PGM3* in PBMCs (upper panels) and SV40 fibroblast (lower panels) P1, P2 controls (C1 and C2), and the patients' father (F) and mother (M). β -Actin and GAPDH were used as loading controls. The panels illustrate results from a single experiment, representative of three. C, Representation of *PGM3* protein, isoform 1, indicating different domains and the position of the variants T464I and Q478X. D, Protein modeling of WT and mutated *PGM3* indicated by green and red arrows' mutations T464I and Q478X

that the variants disturb the structure of the phosphate-binding domain affecting the interaction with substrate (Figure 2C,D). Variants that decrease *PGM3* activity are associated with reduced complex N-glycosylation of T cells, particularly naive $CD4^+$ T cells.²⁸ Accordingly, we measured surface complex N-glycosylation on naive $CD4^+$ T cells using the lectin L-PHA, which binds highly complex N-glycans that include a β 2,6 branch motif. We found that L-PHA binding was substantially reduced in $CD45RA^+$ $CD45RO^-$ naive $CD4^+$ T cells from patients compared to their parents and healthy controls, consistent with reduced *PGM3* activity (Figure 3A).

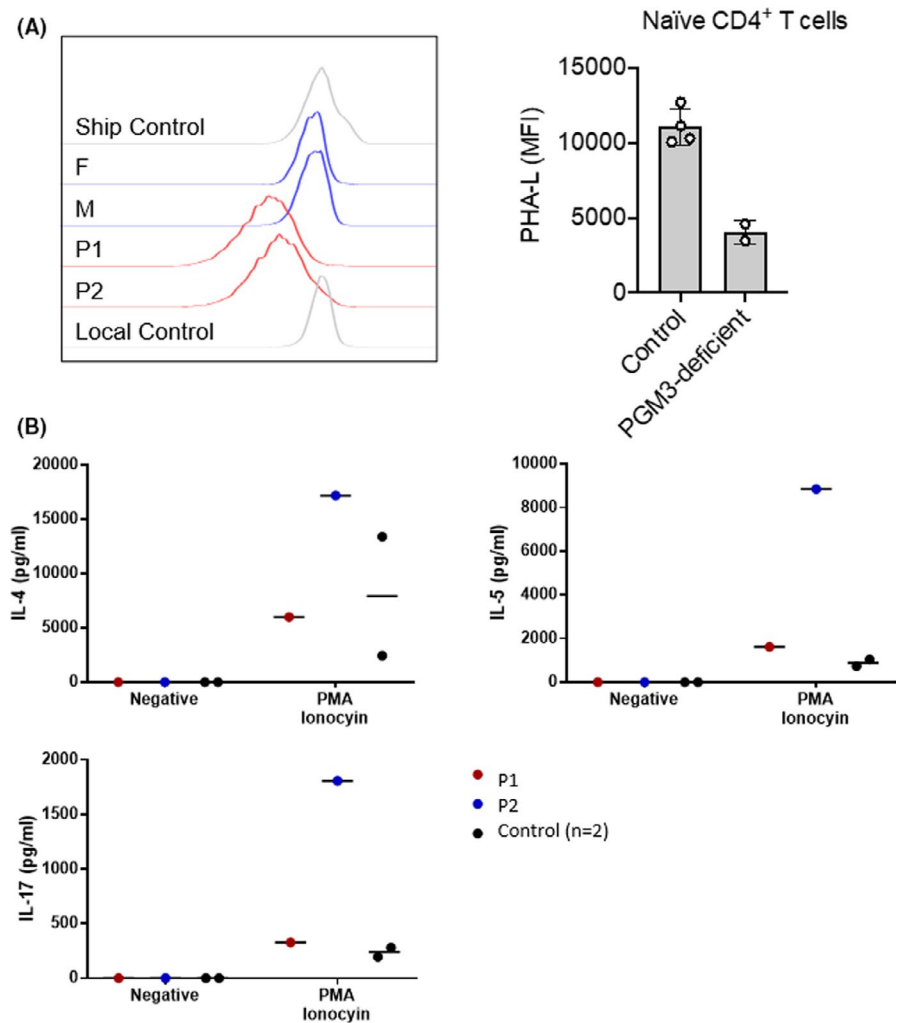
We then evaluated the differential presence of previously described variants in the complex N-glycosylation CDG pathway in both affected individuals in an attempt to explain their phenotypic differences. P1, with the milder phenotype, bears 30 variants with low-to-moderate predicted impact, while P2 showed 43 variants, with 22 of them overlapping with P1 (Figure S2). Interestingly, among the variants found in P2 there were 13 variants in *ALG12*, one of which was a predicted to be damaging with moderate-to-high impact previously reported by Citro *et al* (g50297888, c.1177A > G, p.I393V; rs3922872).⁷ Neither affected sibling carried the previously reported *ALG6* variant (p.L453V, c.1357C > G).⁸

4 | DISCUSSION

We identified two sisters with previously unreported compound heterozygous variants in *PGM3* affecting the phosphate-binding domain of the enzyme. A prominent feature of patients reported previously with *PGM3* deficiency has been syndromic features or neurological impairment. However, these newly identified patients do not show these symptoms.

Monogenic disorders must be suspected in patients presenting with significant elevations in IgE > 2000KUI/L (HIES) and moderate-to-severe eczema, *plus*: 1) recurrent or unexpected infections such as sinopulmonary infections, disseminated molluscum or warts, and mucocutaneous candidiasis; and/or 2) non-immunological syndromic manifestations, such as skeletal and connective tissue disorders, and neurodevelopmental delay.²⁹ Immunologically, T- and B-cell compartments of these patients should be evaluated, including Th17, as depicted in Table 1. These results may help reveal the underlying defect and suggest individualized therapies for patients—such as a need for antibiotic prophylaxis, immunoglobulin replacement treatment, or potentially even hematopoietic stem cell transplantation.³⁰ The warning signs to suspect *PGM3* deficiency among these presentations include hyper-IgE, eosinophilia,

FIGURE 3 A, PHA-L staining of naïve CD4⁺ T cells. Left: histograms showing staining intensity of both a shipped and local healthy volunteer (gray), the unaffected father (F), mother (M), (blue) and two affected individuals (P1 and P2; red); right: geometric mean intensity (MFI) of PHA-L staining of the surface of naïve CD4⁺ T cells. B, IL-4, IL-5, and IL-17 secretion after 16 h of PBMCs culture with and without PMA-ionomycin stimulation of P1, P2 and two healthy controls. Cells were stimulated for 16h with the following conditions: 1) unstimulated, and 2) PMA (50 ng/mL; Sigma-Aldrich) and ionomycin (1 μ L/mL; Sigma-Aldrich); supernatants were collected, and IL-4, IL-5, and IL-17 cytokine secretion was assessed by Luminex (Millipore, Billerica, MA, USA) following manufacturer's instructions



recurrent infections and warts (mucocutaneous candidiasis has not been reported), autoimmunity, elevated IgA, lymphopenia (CD4⁺ and CD8⁺, can range from mild-moderate to severe, with a combined deficiency phenotype²²), and normal-to-increased Th17 cell counts. Interestingly, we observed a considerable increase in IL4 and IL5 response in P2 that could explain her severe atopy manifestations, in comparison with P1 who only presented mild dermatitis (Figure 3B). Most patients have a neurodevelopmental delay and/or skeletal dysplasia (associated with CDG disorders), but not all, as may be seen in the patients reported herein. The limitation of the current diagnosis of PGM3 as a CDG is that transferrin and apolipoprotein CIII isoform analysis in serum are the initial screening tests for suspected CDG (N- and O-glycosylation defects as well as glycan structure analysis) (Figure 1). The main CDG profiles that can be identified with this analysis are type I, some type II, and mixed type CDGs. Characteristically, PGM3 deficiency shows a normal transferrin and apolipoprotein profile (normal values confirmed in both siblings) and thus is suboptimal in confirming the diagnosis. This poses a problem for PGM3 deficiency detection, so genetic confirmation is needed.

To date, some reports have tried to establish a correlation between genotype and phenotype but consanguinity frequently confounds these analyses. In some cases, missense mutations have been

reported with milder phenotypes, but this is not universal, as shown in patients with p.F399L.¹⁹ The role of residual enzymatic activity in the definite phenotype remains to be established. Conversely, the presence of polymorphisms in other proteins involved in N-glycosylation pathways that could alter biological processes, such as polymorphisms in ALG6, has not previously been considered. The lack of information about genotype-phenotype correlations is a relevant limitation in most CDGs, and even more so if we consider that many patients are compound heterozygous. Whether additional hypomorphic variants in other genes involved in N-glycosylation may explain differences in expressivity and severity of disease in PGM3 deficiency deserves further study in larger series using expanded bioinformatics algorithmic approaches for data interpretation. In our two patients, sharing the same PGM3 pathogenic variants, we found an important grouping of polymorphisms in the sibling who presented with more severe disease, particularly in ALG12. If these hypomorphic variants modify N-glycosylation, they might explain some of the differences in clinical and immunological phenotypes of our patients, as has been proposed by previous publications.⁷ However, a difference in complex branching of N-glycans was not observed between patients; this may be the result of the insensitivity of the assay in detecting minor differences (that may be clinically relevant)

or the result of the ALG12 variant not altering N-glycan complexity. Thus, we were not able to determine whether the ALG12 variant is the cause for the different clinical phenotypes observed in these siblings; this could itself open a new line of investigation. Recently, a patient with an ALG12 homozygous variant was described, presenting a mild phenotype³¹; this might support the fact that although P2 (who already has another CDG) does not have a biochemical expression of the ALG12 variant, it could contribute to the differential phenotype of the sisters. This may be extended to other IELs related to glycosylation pathway; indeed, MAGT1-CDG was reported in a family with two girls with mild cognitive impairment and two boys with more severe cognitive involvement, and in XMEN disease (chronic EBV infections attributed to a Mg2+ homeostasis defect), in which no neurological impairment could be observed.²⁵

As for future perspectives in therapy,² a trial providing GlcNAc and uridine oral supplementation to two PGM3-deficient patients has been completed (NCT02511041), and the results are eagerly awaited.

In summary, PGM3 deficiency, as with other CDG, can present with variably expressed phenotypes. The warning signs for suspecting PGM3 deficiency include the combination of hyper-IgE, eosinophilia, recurrent infections and warts, autoimmunity, elevated IgA, lymphopenia, and normal or increased Th17 cells; not all patients will show neurodevelopmental delay and/or skeletal dysplasia. Glycosylated transferrin isoforms will likely be normal. Further research should be focused on defining tools for uncovering genotype/phenotype correlations, as well as on targeted therapies.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

Ana García García: Data curation (supporting); Resources (equal); Visualization (supporting); Writing-original draft (supporting); Writing-review & editing (equal). **Montserrat Buendia Arellano:** Data curation (supporting); Investigation (equal); Visualization (equal). **Àngela Deyà-Martínez:** Data curation (supporting); Resources (equal); Writing-review & editing (supporting). **Jaime Lozano:** Resources (supporting); Writing-review & editing (supporting). **Mercedes Serrano:** Data curation (equal); Resources (supporting); Writing-original draft (equal). **Ana Van Den Rym:** Investigation (equal). **Blanca García-Solís:** Investigation (equal). **Ana Esteve Solé:** Investigation (equal); Visualization (equal); Writing-review & editing (supporting). **Luo Yiyi:** Investigation (supporting); Visualization (equal); Writing-review & editing (supporting). **Alexandru Vlasea:** Investigation (supporting); Writing-review & editing (supporting). **Xavier Solanich:** Writing-review & editing (supporting). **Megan R. Fisher:** Formal analysis (equal); Investigation (equal); Validation (equal). **Jonathan J. Lyons:** Formal analysis (equal); Investigation (equal); Validation (equal); Writing-review & editing (equal). **Rebeca Pérez de Diego:** Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Supervision (lead); Validation (lead); Writing-original draft (equal); Writing-review & editing (supporting). **Laia Alsina:** Conceptualization (lead); Data curation (equal); Funding acquisition (lead); Methodology (supporting); Project administration (equal); Resources (equal); Supervision (lead); Writing-original draft (equal); Writing-review & editing (equal).

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX 1

Description of the methodology used to perform the human molecular genetics and whole exome sequencing (WES).

Genomic DNA was extracted from whole blood with a kit (Qiagen GmbH, Hilden, Germany), used according to the manufacturer's instructions.

Exome sequencing was performed on genomic DNA from whole blood with an Illumina HiSeq 2500 machine and an Agilent 71Mb SureSelect exome kit. The reads were aligned with the human reference genome, using a BWA aligner,³⁴ and then recalibrated and annotated with GATK,³⁵ PICARD (<http://picard.sourceforge.net/>) and ANNOVAR.³⁶ The variants were then filtered and investigated with our in-house online server.

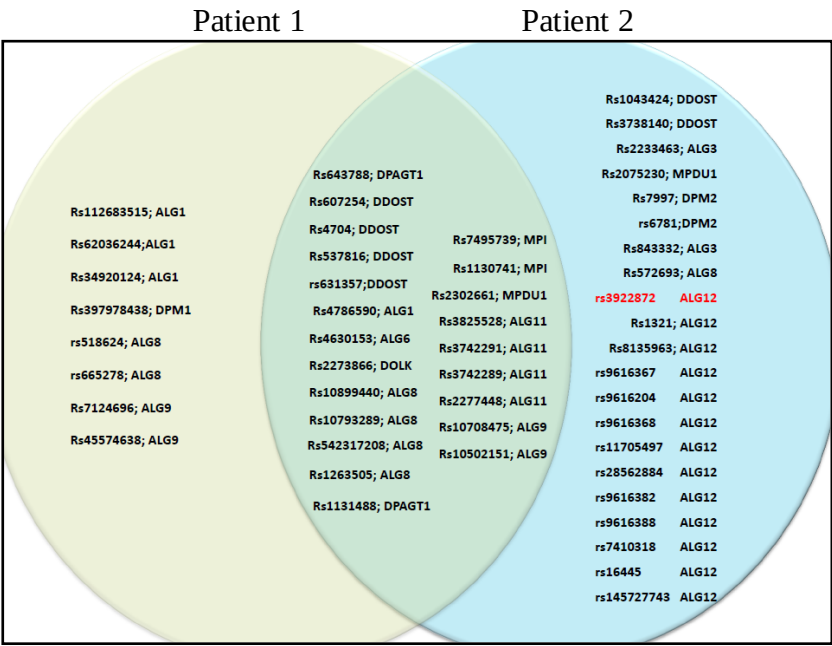
The results of exome sequencing were validated by PCR/sequencing analysis on genomic DNA from whole blood. PCR was carried out with PCR Master Mix (Promega, Fitchburg, WI, USA) and the GeneAmp 9700 PCR System (Applied Biosystems, Foster City, CA, USA). The primer sequences used for the genomic coding region of *PGM3* are shown in Table S2.

The PCR products were purified with the QIAquick gel extraction kit (Qiagen) and sequenced with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing products were purified by precipitation in 70% ethanol, and sequences were analyzed with an ABI Prism 3700 apparatus (Applied Biosystems).

Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Table 1. List of CDG associated with major and minor immunological impairment (9,10).

Major	Minor
ALG12-CDG	ALG1-CDG
ATP6AP1-CDG	ALG14-CDG
EXTL3-CDG	ATP6AP2-CDG
FUT8-CDG	C1GALT1C1-CDG
G6PC3-CDG	COG6-CDG
JAGN1-CDG	DOLK-CDG
MOGS-CDG	GALNT3-CDG
PGM3-CDG	MAN1B1-CDG
SLC35A1-CDG	MGAT2-CDG
SLC35C1-CDG	PMM2-CDG
SLC37A4-CDG	SLC39A8-CDG
	VPS13B-CDG

Supplementary Table 2. Primer sequences used for the genomic coding region of *PGM3*.

Exon	Sequence
PGM3-2-forward	CTATTCGTTTGGGGTGGCAG
PGM3-2-reverse	GACACTAAGGCTTGGTCTTGT
PGM3-3-forward	TCTGTTCTCAACTCAGCCTCA
PGM3-3-reverse	CCTACTAGAAAGGCCAGGCA
PGM3-4-forward	CATTTGCTTGCAGAACCAGC
PGM3-4-reverse	AAACATGCCTGCCCAGAAAC
PGM3-5-forward	AGAAATGCGGTGAATTGCTTT
PGM3-5-reverse	AGGAAAGAGGAGGCATGACA
PGM3-6-forward	ACTGCCCATTGTCTGTGTG
PGM3-6-reverse	TTTGCAGTGCTAGTACGACA
PGM3-7-forward	GTCTAGCCAAACCAACCTGC
PGM3-7-reverse	AGTCTTGTTTTAGAGGGTAGCCA
PGM3-8-forward	TTTGGTAGCCTTTAGAACTCTCA
PGM3-8-reverse	TGAGGGCAGTATCTTTCTCACA
PGM3-9-forward	GCCCCAGTATTTGTTCCCC
PGM3-9-reverse	ATAAAGAAAAGTGCCAGCTAAGG
PGM3-10-forward	CCATTTGTTTCCCCATTGTCAG
PGM3-10-reverse	TCCCTTGACCTCATGATCCG
PGM3-11-forward	AGTCATATCGTATTTACTGGCCA
PGM3-11-reverse	TTGCCTGCCCATCACCTAAT
PGM3-12-forward	GAATCACCCCTCTCTTCCCC
PGM3-12-reverse	AGCCTGCATGAATTGTTCCC

Supplementary Table 3.

From 1976 to date, 40 patients with PGM3 deficiency and from 17 different kindreds, have been

described. Their clinical manifestations are summarized in the table below:

[illegible]

Salmonella septicemia	No	No	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1/1	1/3
Neurological												
DD/Low IQ	No	No	n.d	6/8	6/7	2/3	n.d	n.d	8/9	0/1	1/1	23/31
Autism	No	No	n.d	1/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1/10
Hypotonia	No	No	n.d	1/8	3/7	n.d	n.d	n.d	n.d	n.d	n.d	4/15
Cerebellar symptoms: dysarthria, ataxia	No No	No No	n.d	4/8 7/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	4/10 7/10
Myoclonus	No	No	n.d	5/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	5/10
Seizures	No	No	n.d	2/8	n.d	1/3	n.d	n.d	4/9	n.d	n.d	7/22
CNS myelination defect	n.d.	No	n.d	4/8	0/1	1/1	0/1	n.d	n.d	n.d	n.d	5/12
Hydrocephalus	No	No	n.d	n.d	n.d	2/3	n.d	n.d	n.d	n.d	n.d	2/5
Microcephaly	No	No	n.d	1/8	n.d	0/2	0/1	0/2	n.d	n.d	n.d	1/15
Sensorineural hearing loss	No	No	n.d	4/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	4/10
Prolonged VEP	No	No	n.d	2/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	2/10
Reduced vibratory sensation	No	No	n.d	1/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1/10
Connective tissue												
Dysmorphic facial features	No	Yes ⁸	n.d	4/9	n.d	2/3	1/1	2/2	n.d	0/1	n.d	10/18
Hyperextensibility	No	No	n.d	3/9	0/3	n.d	n.d	n.d	4/9	n.d	n.d	9/23
Skeletal dysplasia	No	No	n.d	n.d	n.d	2/3	1/1	2/2	n.d	0/1	n.d	5/9
Scoliosis	Yes ⁹	No	n.d	4/8	1/9	n.d	n.d	n.d	4/9	0/1	n.d	10/29
Degenerative disc disease	No	No	n.d	1/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1/10
Pectus carinatum	No	No	n.d	n.d	n.d	2/3	n.d	n.d	n.d	n.d	n.d	2/5
Cardiovascular abnormalities	No	No	n.d	1/8	n.d	n.d	1/1	2/2	n.d	1/1	n.d	5/14
Pulmonary												
Bronchiectasis	No	No	n.d	5/8	6/9	n.d	n.d	n.d	n.d	0/1	n.d	11/20
Renal												
MPGN	No ¹⁰	No ¹⁰	n.d	2/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	2/10
Lithiasis	No	No	n.d	1/8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1/10
Kidney malformations	No	No	n.d	1/8	n.d	n.d	1/1	2/2	n.d	n.d	n.d	4/13
Hematological												

Polycythemia	No	No	n.d.	1/8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1/10
Hemolytic anemia	No	No	n.d.	1/8	n.d.	0/3	n.d.	n.d.	n.d.	n.d.	n.d.	1/13
Anemia	No	No	n.d.	n.d.	n.d.	n.d.	1/1	2/2	n.d.	1/1	n.d.	3/6
Thrombocytopenia	No	Yes ¹¹	n.d.	n.d.	n.d.	0/3	0/1	2/2	n.d.	n.d.	n.d.	3/8
Autoimmune neutropenia	No	No	n.d.	1/8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1/10
Other neutropenia	No	No	1/4	n.d.	n.d.	3/3	1/1	2/2	n.d.	n.d.	1/1	8/13
Bone marrow failure	No	No	n.d.	n.d.	n.d.	1/3	0/1	2/2	n.d.	n.d.	n.d.	3/8
Hepatosplenomegaly	No	No	n.d.	1/8	n.d.	0/3	n.d.	1/2	n.d.	0/1	n.d.	2/16
Hodgkin's lymphoma (EBV+)	No	No	n.d.	2/8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2/10
Other autoimmune/autoinflammatory manifestations												
Rheumatoid arthritis	No	No	1/4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1/6
Thyroid autoantibodies	No	No	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1/1	n.d.	1/3
Gastrointestinal and nutritional												
Failure to thrive	No	No	0/2	n.d.	7/9	1/2	n.d.	n.d.	6/9	1/1	n.d.	15/25
GE reflux	No	No	n.d.	n.d.	n.d.	3/3	n.d.	n.d.	n.d.	n.d.	n.d.	3/5
Esophageal stricture	No	No	n.d.	2/8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2/10
Esophageal diverticulum	No	No	n.d.	1/8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1/10
GI obstruction/malrotation	No	No	n.d.	n.d.	n.d.	n.d.	1/1	2/2	n.d.	n.d.	n.d.	3/5
Meckel diverticulum	No	No	n.d.	n.d.	n.d.	n.d.	n.d.	1/1	n.d.	n.d.	n.d.	1/3
Persistent diarrhea	No	No	n.d.	n.d.	n.d.	1/3	1/1	n.d.	n.d.	1/1	1/1	4/8

*Severe combined immunodeficiency patients with PGM3 deficiency are described

**Ben-Khemis describes 9 patients from 2 families (the other family had previously been described by Sassi *et al*)

¹P2 has proved to be clinically allergic to some nuts and banana, but the family refused to do any studies and she is on an exemption diet.

²P2 had a retarded anaphylaxis to omalizumab when the first dose was administered, and although studies are not conclusive, we have not administered further doses.

³ Candida invasive infection refers to pneumonia, otitis, abscess, or septicemia. P1 presented *C.albicans* otomastoiditis.

⁴ Refers to mucocutaneous candidiasis

⁵ Both patients presented negative EBV PCR and negative specific IgG and IgM, without symptomatic infection.

⁶ Both patients had negative CVM PCR and negative specific IgG and IgM.

⁷ Both patients suffered from genital warts caused by HPV-6.

⁸ P2 presented micrognathia.

⁹ P1 presented mild dorsal scoliosis.

¹⁰ Both patients presented normal creatinine, protein and albumin levels throughout the follow-up, without proteinuria. They never had decreased immunoglobulin levels, nor a defect in specific antibody production.

¹¹ P2 had a transient episode of thrombocytopenia at age 8, with nadir counts of 28,000 platelets/mm³ with normal haemoglobin values and no signs of hemolysis, after a viral infection that resolved in 8 weeks and never recurred.

Neither sibling showed clinical or radiological signs of chronic lymphoproliferative disease or autoimmune cytopenias, and nor did they show an increase in double-negative abT cells, and they maintained normal vitamin B12 levels.

P1: Patient 1; P2: Patient 2; t.n.: total patients described; k.n.: number of kindreds; c.k.: consanguinity kindreds; n.d.: no data available; FPIES: Food protein induced enterocolitis; EMM: Erythema multiforme major; EH: Eczema herpeticum; RSV: Respiratory syncytial virus; SSTI: Skin and soft tissue infections; DD: Developmental delay; MPGN: Membranoproliferative glomerulonephritis; GE: Gastroesophageal; GI: Gastrointestinal.