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## Hypomorphic, homozygous mutations in *Phosphoglucomutase 3* impair immunity and increase serum IgE levels

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## Abstract

**Background**—Recurrent bacterial and fungal infections, eczema and elevated serum IgE levels characterize patients with the hyper-IgE syndrome (HIES). Known genetic causes for HIES are mutations in *STAT3* and *DOCK8*, involved in signal transduction pathways. However, glycosylation defects have not been described in HIES. One crucial enzyme in the glycosylation pathway is Phosphoglucomutase 3 (PGM3), which catalyzes a key step in the synthesis of UDP-GlcNAc which is required for the biosynthesis of N-glycans.

**Objective**—To elucidate the genetic cause in HIES patients who do not carry mutations in *STAT3* or *DOCK8*.

**Methods**—After establishing a linkage interval by SNP-chip genotyping and homozygosity mapping in two HIES families from Tunisia, mutational analysis was performed with selector-based, high-throughput sequencing. Protein expression was analyzed by Western blotting and glycosylation was profiled by mass spectrometry.

**Results**—Mutational analysis of candidate genes in a 11.9 Mb linkage region on chromosome 6 shared by two multiplex families identified two homozygous mutations in *PGM3* which segregated with the disease status and followed a recessive inheritance trait. The mutations predict amino acid changes in Phosphoglucomutase-3; PGM3 (p.Glu340del and p.Leu83Ser). A third homozygous mutation (p.Asp502Tyr) and the p.Leu83Ser variant were identified in two other affected families, respectively. These hypomorphic mutations have impact on the biosynthetic reactions involving UDP-GlcNAc. Glycomic analysis revealed an aberrant glycosylation pattern in leukocytes demonstrated by a reduced level of tri-/tetra-antennary N-glycans. T cell proliferation and differentiation was impaired in patients. Most patients showed developmental delay and many had psychomotor retardation.

**Conclusion**—Impairment of *PGM3* function leads to a novel primary (inborn) error of development and immunity, as biallelic hypomorphic mutations are associated with impaired glycosylation and a hyper-IgE-like phenotype.

### Keywords

Hyper-IgE syndrome; glycosylation; Staphylococcus aureus; STAT3; DOCK8; PGM3

## INTRODUCTION

Primary immunodeficiencies (PIDs) are rare diseases, arising from inborn errors of immunity that often follow Mendelian inheritance.<sup>1</sup> To-date, disease-causing mutations in more than 200 genes have been discovered in patients with PID (<http://www.iuisonline.org/iuis/index.php/primary-immunodeficiency-expert-committee.html>). These can affect the expression and/or function of proteins involved in various innate and adaptive immunological processes.<sup>2,3</sup> In patients with one of these PIDs, termed the hyper-IgE syndrome (HIES), monogenic defects have been detected in *STAT3*, *DOCK8* and *TYK2*,<sup>4–8</sup> encoding proteins with pivotal roles in signal transduction pathways in immune cells.

Congenital disorders of glycosylation (CDGs) have been described to lead to PID, since accurate glycosylation of most immune receptors, immunoglobulins, proteins of the complement and cytokines is essential for the integrity of the immune functions.<sup>9–10</sup> Profound hypo-glycosylation of *N*- and *O*-glycans has been associated with *G6PT* mutations in patients with glycogen storage disease type-1b (GSD-1b) and recurrent bacterial infections and with mutations in *G6PC3* in a subset of patients with severe congenital neutropenia.<sup>11–13</sup> Moreover, mutations in the CDG genes *SLC35C1* (CDG-IIc)<sup>14</sup> and *ALG1* (CDG-Ik)<sup>15</sup> cause immune defects. *SLC35C1* deficiency occurs in a PID termed leukocyte adhesion deficiency type II.<sup>16</sup>

The phosphoglucomutases (PGMs) belong to the family of phosphohexose mutases that catalyze the reversible conversion of glucose-1-phosphate (Glc-1-P) to glucose-6-phosphate (Glc-6-P). In contrast, the ubiquitously expressed human phosphoglucomutase 3 (PGM3; identical to phosphoacetylglucosamine mutase 1; AGM1), catalyzes the conversion of GlcNAc-6-P to GlcNAc-1-P, which is required for the biosynthesis of UDP-GlcNAc, an essential precursor for protein glycosylation.<sup>17–18</sup> Hence, deficiencies in PGM3 are likely to impair glycan-mediated processes such as cell-cell recognition or immune signaling. In mice, Pgm3-mediated UDP-GlcNAc synthesis is essential for hematopoiesis and development and distinct recessive hypomorphic *Pgm3* mutations lead to overlapping, but not identical phenotypes.<sup>19</sup>

In this study, we identified mutations in *PGM3/AGM1* in nine patients from four consanguineous families with recurrent infections, elevated IgE in serum, but with normal *STAT3* and *DOCK8*. Biallelic hypomorphic *PGM3* mutations were associated with impaired glycosylation due to impaired PGM3 function and thus characterize a novel group of primary (inborn) immune deficiency with a hyper-IgE-like syndrome.

## METHODS

### Patients and controls

This study was conducted under human subjects' protocols approved by local ethics committees at University College London, the University of Freiburg, the Pasteur Institute of Tunis, Erciyes University, Turkey, and Hassan II University, Morocco. Six HIES patients with an autosomal recessive inheritance pattern from two Tunisian families (A and B), one patient from a Turkish family (C) and two patients from a Moroccan family (D) were the focus of this study. Further 30 affected individuals were tested for *PGM3* mutations. unaffected individuals, originating from Tunisia (100), Morocco (20) and Turkey (50), served as controls. The race or ethnic group of the Tunisian control subjects was self-reported and considered as "North African". Written consent was given by study participants and/or their parental guardians, following local ethics committee requirements.

### Methods

Supplemental information can be found in the Methods section in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

## RESULTS

### Clinical assessments of affected individuals

All patients in this study have been independently diagnosed with HIES based on the clinical triad of recurrent pneumonia, recurrent skin abscesses and a highly elevated serum IgE. Family B has been previously described as suffering from Buckley syndrome, a synonym for hyper-IgE syndrome.<sup>20</sup> Clinical and laboratory findings, B- and T-cell phenotyping and T-cell proliferation are summarized in Tables I – IV and Figures E1 and E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). In summary, eosinophilia and an inverted CD4/CD8-ratio, in addition to the elevation of serum IgE, were characteristic laboratory findings in our patients. As known for other CDG disorders, most routine laboratory values were not consistently altered in all patients. Remarkably, most *PGM3* patients showed developmental delay and many had psychomotor retardation, resembling clinical findings in CDG.

### Identification of *PGM3* mutations by homozygosity mapping/linkage analysis and selector-based sequencing

Using a positional approach to identify the disease causing mutations in both Tunisian families A and B (Fig 1, *A–B*), we identified overlapping perfect intervals on chromosome 6q. Family B had a huge perfect interval spanning chr6:54.3–107.0Mbp (build 36/hg18 positions) and family A had its largest perfect interval at chr6:73.2–85.1Mbp. Perfectly segregating intervals in these families yielded LOD scores of at least 3.32 for family A and 1.65 for family B, with a multipoint LOD score of at least 4.97. We thus hypothesized that they might have the same monogenic disease, but, because of the more severe phenotype in family A, not necessarily the same mutation. The intersection of the two intervals, chr6:73.2–85.1Mbp, contained 45 candidate genes (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) and both families shared a small disease-associated haplotype at chr6:82.88–83.40Mbp. However, in a candidate approach, comprising several

genes associated with immune functions, mutations in the coding exons or flanking intronic regions were not detected.

We therefore performed selector-based<sup>21</sup>, high-throughput sequencing of all coding exons, exon/intron boundaries and untranslated regions of all 45 genes in the predicted region. We identified two sequence variants in *PGM3* (ENSP00000424874/PGM3-001/NP\_056414), which spans 29 kb, comprises 14 exons and encodes phosphoclucomutase-3 (PGM3). Exon 3 contains the start codon for transcript variant 1 which encodes the 542 amino acid PGM3 isoform 2 (see supplementary information in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Both mutations affect highly conserved amino acid residues and predict a one amino acid deletion p.Glu340del (c.1018\_1020del; exon 9) in family A (Fig 1, E), and a substitution p.Leu83Ser (c.248T>C; exon 4) in family B (Fig 1, F), respectively. Modeling the p.Leu83Ser mutation *in silico* predicted the most deleterious effect (PolyPhen<sup>22</sup> prediction: probably damaging; SIFT<sup>23</sup> score 0.00).

We next sequenced *PGM3* in 32 unrelated patients, with phenotypes resembling AD- or AR-HIES but without mutations in *STAT3* or *DOCK8*. In the affected individual of a consanguineous Turkish family (Family C; Fig 1, C,G) we identified a third homozygous point mutation in *PGM3* (p.Asp502Tyr; c.1504G>T; exon 13), which is also predicted to be damaging by both PolyPhen and SIFT (score 0.00). Furthermore, the HIES index patient from Morocco and an affected sibling (Family D; Fig 1, D,H) carry the same mutation p.Leu83Ser (c.248T>C) as identified in family B. Genotype data on family D, to evaluate whether families B and D have a common ancestor, are not available.

The *PGM3* mutations (see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) segregate perfectly with the disease phenotype in each family (Fig 1). The variants were absent in 170 control individuals from Tunisia (100), Turkey (50) and Morocco (20), excluding that the novel mutations are polymorphisms in these populations.

### Impaired PGM3 expression in affected individuals carrying homozygous p.Glu340del mutations

PGM3 comprises four functional domains (Fig 2, A). The identified mutations are located in the N-terminal catalytic (p.Leu83Ser; Families B and D), the central sugar binding (p.Glu340del; Family A), and the C-terminal PO<sub>4</sub><sup>-</sup>-binding (p.Asp502Tyr; Family C) domains of the protein, respectively. Thus, each of the identified mutations predicts the loss of a unique protein function. To test whether PGM3 expression was impaired, we analyzed lysates from immortalized B- or T-cells derived from patients and unaffected members from families A, B and C, and from control subjects by Western blotting (Fig 2, B–C). PGM3 was expressed at normal levels in patients B.V.7 (homozygous for p.Leu83Ser) and C.IV.7 (homozygous for p.Asp502Tyr) as well as in all analyzed heterozygous carriers, whereas patients A.V.12 and A.V.18 (both homozygous for p.Glu340del) showed markedly decreased expression. The latter suggest that the homozygous mutation in Family A, affecting the sugar binding domain, not only causes protein dysfunction but probably also leads to increased protein degradation.

### Mutant PGM3 shows reduced catalytic activity but retains its specificity

The assembly of mono-, bi- tri and tetra-antennary branched N-glycans is dependent on the supply of a common substrate UDP-GlcNAc. Because PGM3 catalyzes the bidirectional conversion of GlcNAc-6-P to GlcNAc-1-P, which is required for the synthesis of UDP-GlcNAc, we hypothesized that impaired PGM3 functions leads to aberrant N-glycosylation (Fig. 3, A). However the *PGM3* mutations in our patients (A.V.12 and C.IV.7), did not affect the glycosylation pattern of transferrin in the blood (see Fig E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), which is a commonly used diagnostic test for congenital defects of glycosylation.<sup>24</sup> Enzymatic testing revealed that the catalytic activity is reduced in mutant PGM3, dependent on the substrate dose, with 20–30% of residual activity (Fig. 3, B). The effect was more pronounced with the p.Glu340del (Family A;  $p < 0.0005$  at 12.5  $\mu\text{M}$  of GlcNAc-6-P) than with the p.Leu83Ser (Family B;  $p < 0.002$ ) mutation. We concluded that the mutant PGM3 retains its catalytic specificity albeit with impaired enzymatic activity. In the accompanying article, a biochemical defect was shown by analyzing intra-cellular UDP-GlcNAc levels (Zhang et al.).

### PGM3-mutations inhibit the formation of complex N-glycan subtypes

Using a common substrate (UDP-GlcNAc), the GlcNAc transferases I (GlcNAc T-I:  $K_m$  0.04) and T-II ( $K_m$  0.96) initiate the first and second N-glycan antennae, whereas T-IV ( $K_m$  5.0) and T-V ( $K_m$  11.0) are required for the third and fourth N-glycan antennae, respectively (Fig 4, A). Due to the higher  $K_m$  value (which in Michaelis-Menten kinetics corresponds to low substrate affinity and thus a low catalytic activity), the initiation of the third and fourth antenna by GlcNAc T-IV and GlcNAc-T V requires higher substrate concentrations and will be most impaired if the level of the common substrate is aberrantly low.<sup>25</sup>

Since PGM3 is involved in the production of UDP-GlcNAc, and mutations in PGM3 therefore might alter the N-glycomes in affected patients, we performed glycomic profiling of neutrophils from patients B.V.7 (p.Leu83Ser) and C.IV.7 (p.Asp502Tyr). In both patients we observed decreased levels of tri- and tetra-antennary N-glycans compared to controls (Fig 4, B; and Fig E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Simultaneously, in patient B.V.7 and to a lesser extent in patient C.IV.7, bi-antennary N-glycan types accumulated. We also examined EBV-transformed B cells from patients A.V.13 (p.Glu340del) and B.V.7 (p.Leu83Ser) and observed a similar reduction in complex-type tri- and tetra-antennary glycans (Fig 4, C; and Fig E5 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The patient from family A showed a substantial accumulation of the hybrid glycans, whereas the bi- tri- and tetra-antennary glycans were accordingly decreased. Thus, the p.Glu340del mutation in Family A predominantly blocks the formation of bi-antennary N-glycans. In contrast, in patient B.V.7, only tri- and tetra-antennary glycans were reduced. Thus, because glycosylation appears to be retarded at the bi-antennary stage, normal levels of hybrid glycans were observed, consistent with the suggested lower severity of the mutation (p.Leu83Ser).

In summary, the homozygous mutation in the sugar binding domain (p.Glu340del in Family A) leads to reduced PGM3 abundance and impairs PGM3 function and glycosylation to a



higher extent than mutations in the catalytic or phosphate binding domain, respectively, and as a consequence, causes a more severe clinical phenotype.

Elevated serum IgE in *PGM3* patients might be caused by increased IgE stability due to aberrant glycosylation.<sup>26</sup> However, no significant differences were observed when IgE N-glycan profiles from a *PGM3* patient and a patient with atopic dermatitis were compared (see Fig E6 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Normal chemotaxis and surface expression of GPI anchored proteins on granulocytes

To test whether the *PGM3* mutations influence the expression of surface molecules, which strongly dependent on the presence of membrane anchors (Glycosyl-phosphatidylinositol (GPI)-anchored proteins), we analyzed the expression of CD59, CD55(DAF), CD24, CD14 and FLAER (Fluorescent labelled aerolysin binding tightly and specifically to mammalian GPI anchors) on granulocytes from patients B.V6 and B.V.7 by flow cytometry (see Fig E7 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). However, we could not demonstrate that the p.Leu83Ser mutation alters the expression of the candidate surface proteins due to defective glycosylation of their membrane anchors.

To test whether the impaired glycosylation affects neutrophil function, we analyzed neutrophil chemotaxis in two Tunisian patients from family B and the Turkish patient after staining with Calcein-AM and stimulation with fMLP, PA and C5a, using a transwell assay. However, when measuring fluorescence intensities on a PerkinElmer EnVision reader, we did not find evidence that chemotaxis is altered in patients compared to travel controls (data not shown).

### Impaired T cell proliferation, Th2 skewing, and borderline Th17 cell numbers in patients with *PGM3* mutations

To test whether the HIES phenotype in our patients with *PGM3* mutations is associated with impaired T cell function, we analyzed freshly isolated PBMC by FACS analysis for proliferation and T cell-specific cytokine responses after stimulation *ex vivo* (Table III and IV and see Fig E1 and E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org); see also the accompanying article by Zhang et al.). T cell proliferation to recall antigens (PPD and tetanus toxoid) was reduced in all samples tested (Table IV). Interestingly, T cell proliferation following stimulation with PHA or anti-CD3 of family B carrying the missense mutation (p.Leu83Ser) was comparable to healthy controls; however, proliferation in family A, with the more severe mutation (p.Glu340del), was again borderline low.

Serum IgE elevation may be explained by abnormal T helper cell differentiation. We therefore analyzed Th1–Th2 subsets in our patients by intracellular IL4 and IFN $\gamma$  staining (Table III; see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Two out of four patients tested had a grossly elevated percentage of Th2 cells (19% and 27%, respectively), likely contributing to the elevated serum IgE in these patients.

In contrast to the other patients, B cells were absent in C.IV.7 and an unusual subpopulation of CD4<sup>+</sup> cells was detected, simultaneously expressing Th1 and Th2 cytokines as well as a reduced proportion of FoxP3 expressing regulatory T cells (data not shown).

STAT3-deficient HIES is characterized by the absence of Th17 cells.<sup>27</sup> In our PGM3 patients we found borderline low Th17 cell numbers (Table III), with a lower IL17 PMA/iono stimulation index when compared to healthy family members. This observation may contribute to the increased infection susceptibility of the skin and mucous membranes in patients with *PGM3* mutations.

## DISCUSSION

We report on homozygous mutations in *PGM3* leading to aberrant glycosylation patterns in leukocytes. Mutations in *PGM3* have previously not been associated with human disease. Here we demonstrate that hypomorphic mutations in *PGM3* constitute a novel genetic defect in patients previously diagnosed as autosomal-recessive HIES (AR-HIES).

*PGM3* has previously been shown to contain a polymorphism with a high minor allele frequency, useful for population genetic studies.<sup>28</sup> The mutations described herein, including two non-synonymous point mutations and a single codon deletion in nine patients from four unrelated families, respectively, have not been identified in other ethnic groups and are not listed in the Human Gene Mutation Database (HGMD) and the 1000 Genomes project. They affect all four known *PGM3* isoforms and localize to three of the four functional protein domains, thus predicting the loss of distinct protein functions.

All patients in our study were independently diagnosed with HIES in three clinical immunology centers solely on the basis of their clinical presentation, including elevated serum IgE, recurrent skin and pulmonary infections, abscesses, eczema, and bronchiectasis. In a previous study<sup>20</sup>, two patients (B.V.6 and B.V.7) were reported as having Buckley syndrome which is an alternative name for HIES. Criteria to distinguish patients with biallelic *PGM3* mutations from other HIES patients might be a reversed CD4/CD8 ratio and a developmental delay.

AR-HIES often occurs in consanguineous families and approximately 80% of affected individuals carry a mutation in *DOCK8* (Engelhardt *et al*; manuscript in preparation). Although the clinical presentations are similar, only one of the nine *PGM3* patients had severe chicken pox and none had a food allergy; in contrast, severe cutaneous viral infections and food allergies occur in 70% of cases with *DOCK8* deficiency.<sup>6,7</sup> In addition, failure to thrive is more prevalent in individuals with mutations in *PGM3* (89%) than *DOCK8* (58%) and frequently coincided with developmental delay and psychomotor retardation, reminiscent of congenital disorders of glycosylation (CDG), but not of *DOCK8* deficiency. Phenotypic discrimination between HIES patients with *STAT3* and *PGM3* mutations is less apparent and includes pneumatoceles and dental abnormalities, which occur in *STAT3*-associated AD-HIES,<sup>5</sup> but not in affected *PGM3* individuals. However, connective tissue and skeletal abnormalities, joint hyperextensibility, scoliosis and a characteristic face occur in both. Thus, our results indicate that *PGM3* mutations characterize a novel subtype of AR-HIES.

In Family A, the p.Glu340del mutation not only led to the most severe pathology and reduced *PGM3* abundance but also blocked the formation of bi-antennary N-glycans. In



contrast, in Family B (p.Leu83Ser) and Family C (p.Asp502Tyr) the bi-antennary N-glycans accumulated and the neurological disorders were milder. This observation is consistent with a genotype-phenotype correlation. In support of this view, an accompanying article (Zhang et al.) describes additional HIES patients from two families carrying distinct PGM3 mutations. All patients presented with bronchiectasis, eczema, abscesses and developmental delays, as observed in our cohort. However, these patients have CD8 lymphopenia, high cholesterol, and renal and liver changes that are not present in our PGM3 patients.

It has recently been reported that *Pgm3*-deficiency causes embryonic lethality in mice<sup>19</sup> whereas *Pgm3* gene trap mice showed reduced body weights, defective B-lymphocyte development and neurodegeneration due to decreased UDP-GlcNAc levels in the brain. Accordingly, in the majority of patients with *PGM3* mutations, we observed a failure to thrive, decreased B-cell numbers and, in some patients, a developmental delay. In contrast, increased numbers of platelets and eosinophilic granulocytes and a reverted CD4/CD8 ratio were not observed in *Pgm3*-mutant mice.

In Congenital Disorder of Glycosylation type II (CDG2A; OMIM#212066) mutations in *MGAT2* (encoding GlcNAc T II which operates downstream of PGM3) cause severe multi-systemic developmental anomalies and reduced abundance of bi-, tri- and tetra-antennary glycans.<sup>29-31</sup> The similarities to patients with *PGM3* mutations indicate that complex N-glycans are essential for normal neuronal development. In congenital dyserythropoietic anemia type II (CDA-II; OMIM#224100) glycomic analysis of erythrocytes showed increased levels of immature hybrid glycans and a marked reduction of branched mature glycans<sup>32</sup> similarly as we observed in the leukocytes of our patients.

Since we did not find convincing evidence for defective N-glycosylation or decreased turnover of IgE, the exact cause for elevated serum IgE levels in patients with *PGM3* mutations, as for other forms of HIES, remains to be determined, although we have demonstrated an abnormal Th1/Th2 differentiation in two of four patients tested. We therefore have to conclude that the pathogenic mechanism of how the aberrant glycosylation leads to the observed HIES-like phenotype remains elusive.

However, the observation that inheritable defects of glycosylation can lead to a HIES-like phenotype has not been recognized previously.<sup>33-35</sup> Although mutations in *PGM1* (encoding phosphoglucomutase 1) can cause a CDG-like phenotype, neurological or immunological disorders as in our PGM3 patients were not observed.<sup>36</sup> Certainly, besides defective glycan biosynthesis, other pathogenic mechanisms such as abrogated membrane transport or gain of glycosylation sites can also account for immune deficiencies.<sup>37-38</sup> Whether hematopoietic stem cell transplantation, a successful treatment option in DOCK8-deficiency,<sup>39</sup> is also effective for patients with *PGM3* mutations has not yet been confirmed.

Our observation, that congenital glycosylation defects can be associated with a rare HIES-like primary immunodeficiency raises the possibility that the prevalent IgE-mediated allergy and atopy seen in these patients might also, at least in part, be due to aberrant glycosylation.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations used

<b>CDG</b>	congenital disorder of glycosylation
<b>DOCK8</b>	dedicator of cytokinesis 8
<b>EBV</b>	Epstein-Barr virus
<b>Glc-1-P</b>	glucose-1-phosphate
<b>Glc-6-P</b>	glucose-6-phosphate
<b>GlcNAc</b>	N-acetyl-D-glucosamine
<b>GlcNAc-1-P</b>	N-acetyl-D-glucosamine-1-phosphate
<b>GlcNAc-6-P</b>	N-acetyl-D-glucosamine-6-phosphate
<b>HIES</b>	Hyper-IgE syndrome
<b>LOD</b>	logarithm of odds
<b>OMIM</b>	Online Mendelian Inheritance in Man
<b>PGM3</b>	phosphoglucomutase 3
<b>PID</b>	primary immunodeficiency
<b>STAT3</b>	signal transducer and activator of transcription 3
<b>Tyk2</b>	tyrosine kinase 2
<b>UDP-GlcNAc</b>	Uridine diphosphate N-acetylglucosamine

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**Clinical implications**

Hypomorphic mutations in *PGM3* are associated with defects in immunity and development in a novel form of primary immunodeficiency with impaired cell- and tissue-specific glycosylation.

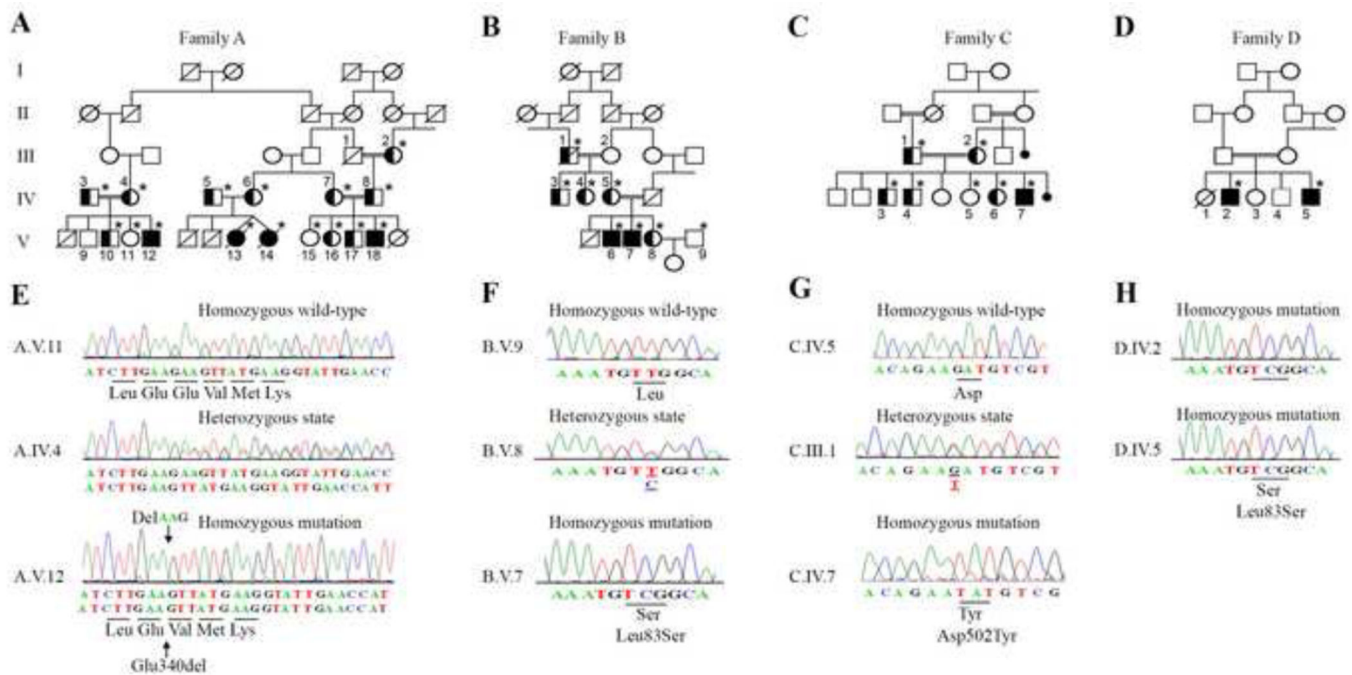
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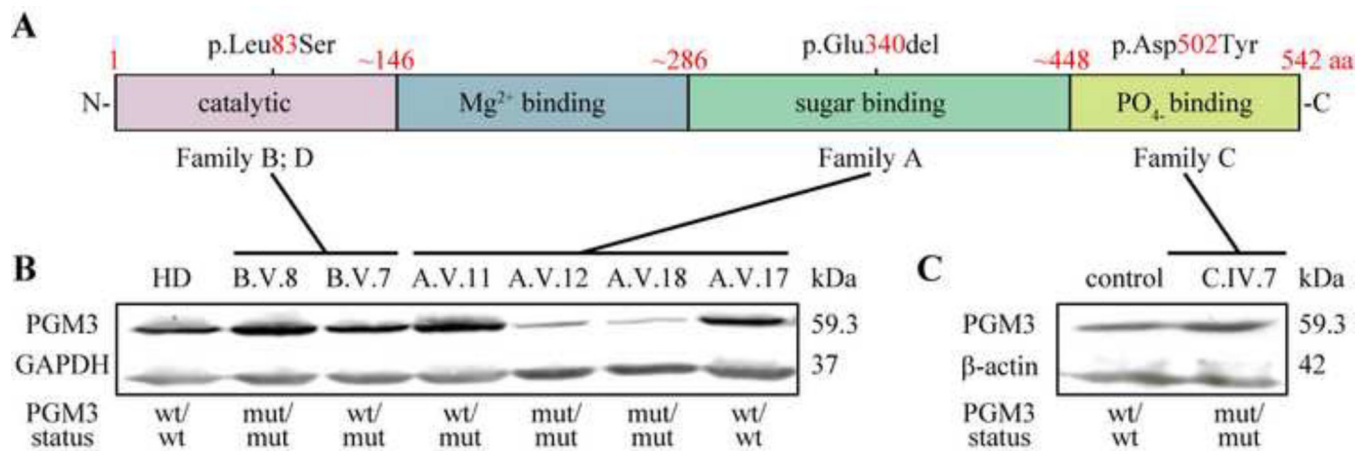
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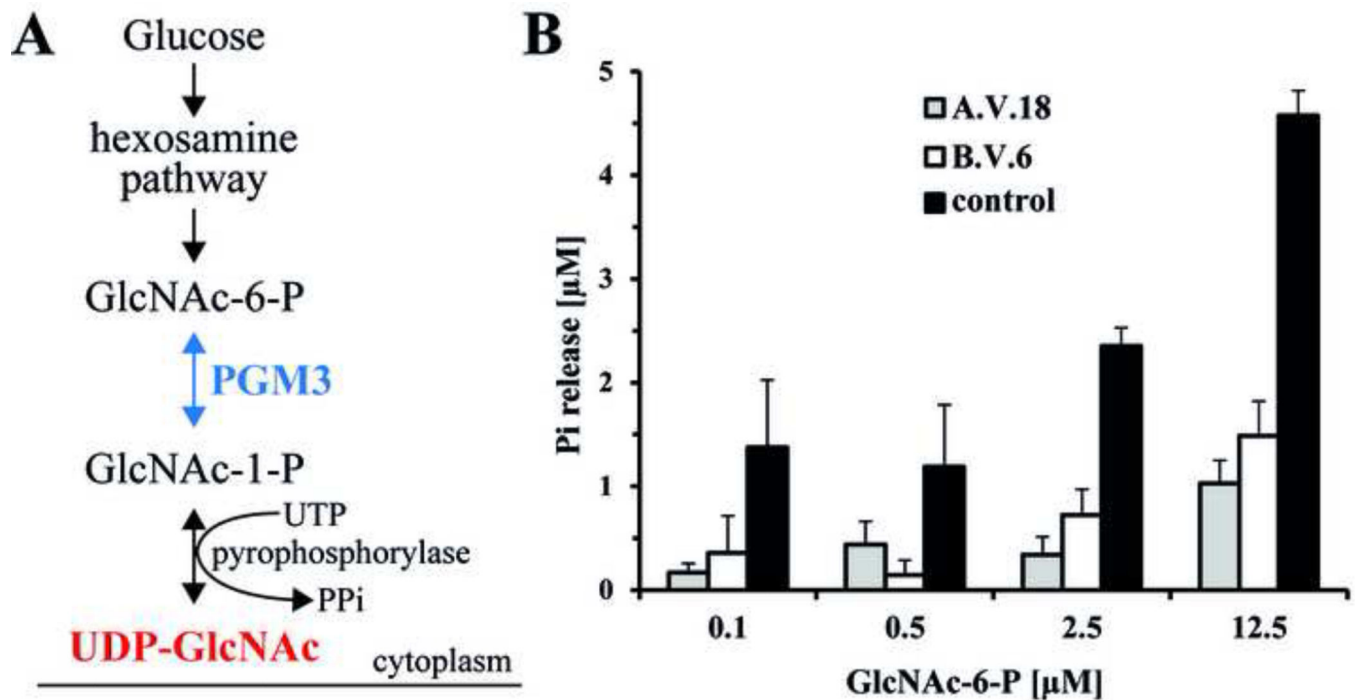


**FIG1.**

Segregation of *PGM3* mutations with the disease status in AR-HIES families. **A–D**. Family A, p.Glu340del; Families B and D, p.Leu83Ser; and Family C, p.Asp502Tyr. Circles, female; squares male; filled symbols, affected individuals with homozygous mutations; half-solid symbols, heterozygous carriers; open symbols, healthy members with wild-type *PGM3*; slashes, deceased individuals; double horizontal lines, consanguinity, black dots, miscarriages. Asterisks, mutations confirmed by sequencing. **E–H**, Sequence analyses of unaffected individuals without *PGM3* mutations (top), unaffected heterozygous carriers (middle panel in E–G) and homozygous affected individuals (bottom).

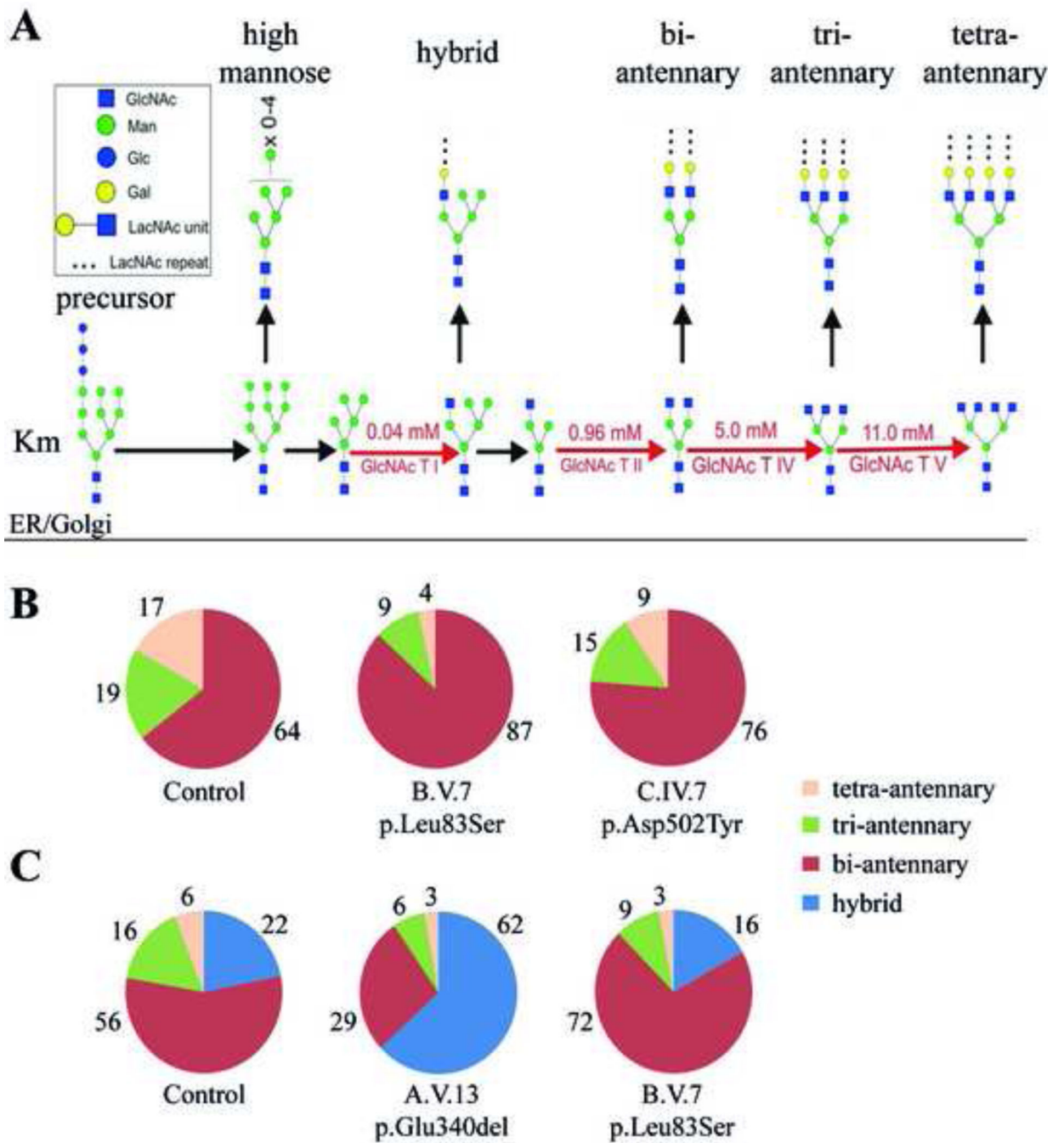
**FIG 2.**

Homozygous p.Glu340del mutations within the sugar-binding domain cause reduced PGM3 expression. **A**, The identified mutations localize to distinct protein domains. Numbers indicate amino acid residues. **B**, Western blotting shows reduced PGM3 abundance in EBV-transformed B cells from both homozygous carriers of family A. HD, healthy donor; the mutational status is indicated below each lane. GAPDH confirms equal loading. **C**, Normal PGM3 expression in immortalized T cells from the patient in family C who had insufficient B cell numbers.  $\beta$ -actin, loading control.



**FIG 3.**

Impaired enzymatic activity in mutant PGM3. **A**, PGM3 (blue) catalyzes the change from GlcNAc-6-P to GlcNAc-1-P, which is required for synthesis of UDP-GlcNAc (red). UDP-GlcNAc is the common substrate for assembly of N-glycans. **B**, Significantly decreased production of inorganic phosphate in EBV-B cell lysates derived from patients A.V.18 (p.Glu3401del;  $p < 0.0005$  at  $12.5 \mu\text{M}$ ) is more pronounced than from patient B.V.6 (p.Leu83Ser;  $p < 0.002$  at  $12.5 \mu\text{M}$ ). Compared to the control ( $n=2$ ), residual, substrate-dependent activity (20–30%) of PGM3 is observed. Data were calculated from three independent experiments, and standard deviations are indicated by error bars.  $P$ -values were calculated using Student's  $t$ -test.



**FIG 4.** Incomplete assembly of complex type glycans in patients with *PGM3* mutations. **A**, *PGM3* links to N-glycosylation through UDP-GlcNAc (red). The common substrate for assembly of the complex type N-glycans in the Golgi (vertical arrows) is used by four GlcNAc Transferases (T-I, -II, -IV and -V; red arrows) with decreasing catalytic activity (increasing  $K_m$  values, mM UDP-GlcNAc)<sup>25</sup> UDP-GlcNAc produced by mutant *PGM3* might be insufficient to allow for completion of N-glycan antenna formation. **B**, Accumulation of bi-antennary N-glycans in neutrophils<sup>41</sup> from patients with *PGM3* mutations. Decreased

relative abundance of tri- and tetra-antennary glycans (percentage values of N-glycans detected by glycomic profiling; see Fig E4, E5 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) in affected individuals B.V.7 (p.Leu83Ser; catalytic domain) and to a lesser extent in C.IV.7 (p.Asp502Tyr; phosphate-binding domain). **C**, The homozygous PGM3 mutation pGlu340del causes accumulation of the hybrid-type N-glycans, with simultaneous decrease of bi-, tri- and tetra- antennary N-glycans in EBV B-cells from patient A.V.13 (p.Glu340del; sugar binding domain). B-cells from patient B.V.7 (p.Leu83Ser; catalytic domain) have decreased tri- and tetra- antennary N-glycans, and increased bi-antennary N-glycans. Percentage values indicate the relative abundance of glycan types.

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TABLE I

Clinical findings in HIES patients with homozygous *PGM3* mutations

Patient	A.V.12	A.V.13	A.V.14	A.V.18	B.V.6	B.V.7	C.IV.7	D.IV.2	D.IV.5
Origin	Tunisia	Tunisia	Tunisia	Tunisia	Tunisia	Tunisia	Turkey	Morocco	Morocco
Sex	m	f	f	m	m	m	m	m	m
Age of onset	4 mo	7 mo	2 mo	1 mo	3 mo	4 mo	6.5 yrs	3 mo	3 mo
Age at last evaluation/death	7 yrs	deceased: 14 mo	deceased: 13 mo	6 yrs 2 mo	34 yrs	32 yrs	11.5 yrs	21 yrs	11 yrs
<i>PGM3</i> mutation	p.E340del	p.E340del	p.E340del	p.E340del	p.L83S	p.L83S	p.D502Y	p.L83S	p.L83S
<i>Symptoms typical for HIES</i>									
Recurrent RTI	y	y	y	y	y	y	y	y	y
Pneumonias	3	3	3	2	>3	>3	3	>3	3
Lung abnormalities (bronchiectasis)	y	n	n	n	y	y	y	y	y
Eczema	moderate	severe	severe	severe	severe	severe	n	severe	n
Abscesses	y	y	y	y	y	y	n	y (cold)	n
Candidiasis	oral	oral	oral	ear	fingermails	oral	n	n	n
Viral infections	n	RSV	RSV	n	n	n	severe VZV	y	n
<i>S. aureus</i> infections	y	y	y	y	y	y	y	y	n
Characteristic facies	n	n	n	n	y	y	n	y	y
Hyper-extensibility	n	n	n	y	y	y	n	n	n
Serum IgE (IU/ml)	18,730	7,174	3,029	16,534	99,600	141,300	9,320	>17,000	>18,500
Eosinophilia	y	y	y	y	y	y	y	y	y
HIES score	50	45	48	47	56	53	27	55	43
<i>Symptoms found in both HIES and CDG</i>									
Failure to thrive	y	y	y	y	y	y	n	y	n
Scoliosis	y	n	n	n	n	n	n	n	n
<i>Symptoms typical for CDG</i>									
Developmental delay	y	N/A	N/A	y	mild	mild	y	mild	n
Psychomotor retardation	y	N/A	N/A	y	n	n	n	y	n



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Patient	A.V.12	A.V.13	A.V.14	A.V.18	B.V.6	B.V.7	C.IV.7	D.IV.2	D.IV.5
Hypotonia	severe	N/A	N/A	severe	n	n	n	y	n

m, male; f, female; yrs, years; mo, months; y, yes; n,no; N/A – not applicable

**TABLE II**

Laboratory findings in HIES patients with homozygous *PGM3* mutations

	A.V.12	A.V.13	A.V.14	A.V.18	B.V.6	B.V.7	C.IV.7
Age at measurement	7y	11mo	11mo	6y	7y	6y	9y
<b>Cell counts/<math>\mu</math>l</b>							
WBC	6,300 (4,400–9,500)	11,600 (6,400–13,000)	4,600 ↓ (6,400–13,000)	17,060 ↑ (5,200–11,000)	12,100 ↑ (4,400–9,500)	16,100 ↑ (5,200–11,000)	2,280 ↓ (4,400–9,500)
ANC	1,510 ↓ (1,700–7,000)	6,100 (2,000–8,000)	950 ↓ (2,000–8,000)	12,610 ↑ (1,700–7,000)	6,410 (1,700–7,000)	9,660 ↑ (1,700–7,000)	1,090 (1,000–5,000)
ALC	3,530 (1,900–3,700)	4,100 (3,400–9,000)	1,940 ↓ (3,400–9,000)	2,630 (2,300–5,400)	3,150 (1,900–3,700)	3,500 (2,300–5,400)	760 ↓ (1,900–3,700)
AEC	760 ↑ (100–600)	1,540 ↑ (50–600)	700 ↑ (50–600)	510–1,410 ↑ (160–800)	800–1,500 ↑ (100–600)	1,450–2,000 ↑ (160–800)	900 ↑ (100–800)
PLT [x 1000]	nd (130–400)	230 (130–400)	150 (130–400)	606 ↑ (130–400)	600 ↑ (130–400)	670 ↑ (130–400)	199 (130–400)
<b>Lymphocyte subsets %</b>							
CD3	69.5 (60–76)	26 ↓ (49–76)	74 (49–76)	53 ↓ (56–75)	35 ↓ (60–76)	71 (56–75)	87.9 ↑ (60–76)
CD4	13.5 ↓ (31–47)	8 ↓ (31–56)	18.5 ↓ (31–56)	14.5 ↓ (28–47)	29 ↓ (31–47)	25 ↓ (28–47)	46.2 (31–47)
CD8	56.5 ↑ (18–35)	34 ↑ (12–24)	53 ↑ (12–24)	41 ↑ (16–30)	42 ↑ (18–35)	42 ↑ (16–30)	41.2 ↑ (18–35)
CD4/CD8	0.24 (1.34–1.72)	0.24 (2.33–2.58)	0.35 (2.33–2.58)	0.35 (1.57–1.75)	0.69 (1.34–1.72)	0.6 (1.57–1.75)	1.12 (1.34–1.72)
CD19	9 ↓ (13–27)	18.5 (14–37)	8.5 ↓ (14–37)	8.5 ↓ (14–33)	20 (13–27)	42 ↑ (14–33)	1 ↓ (13–27)
NK (CD3– CD16+ CD56+)	25 ↑ (4–17)	52.5 (3–15)	18.5 ↑ (3–15)	47 ↑ (3–17)	14 (4–17)	46 ↑ (3–17)	5.5 (4–17)
<b>Lymphocyte subsets counts/<math>\mu</math>l</b>							
CD3	2,450 (2,120–2,680)	1,066 ↓ (2,000–3,120)	1,435 ↓ (2,000–3,120)	1,400 ↓ (1,470–1,970)	1,100 ↓ (2,120–2,680)	2,490 ↓ (1,470–1,970)	670 ↑ (456–580)
CD4	476 ↓ (1,100–1,700)	328 ↓ (1,270–2,300)	360 ↓ (1,270–2,300)	381 ↓ (740–1,220)	913 ↓ (1,100–1,700)	875 ↓ (740–1,220)	350 (240–360)
CD8	1,994 ↓ (635–1,235)	1,394 ↑ (492–984)	1,030 ↑ (492–984)	1,080 ↑ (416–790)	1,320 ↑ (635–1,235)	1,470 ↑ (416–790)	313 ↑ (140–266)

	A.V.12	A.V.13	A.V.14	A.V.18	B.V.6	B.V.7	C.IV.7
CD19	318 ↓ (460–950)	760 (574–1,520)	165 ↓ (574–1,520)	224 ↓ (370–870)	630 (460–950)	1,470 ↑ (370–870)	7.6 ↓ (100–205)
NK (CD3– CD16+ CD56+)	880 ↑ (141–600)	2,150 ↑ (123–615)	360 (123–615)	1,240 ↑ (80–450)	441 (141–600)	1,610 ↑ (80–450)	42 (23–130)
<b>Serum immunoglobulins</b>							
IgM g/l	1.74 (0.75–1.97)	2.25 ↑ (0.4–1.9)	3.93 ↑ (0.4–1.9)	0.698 (0.4–2)	1.45 (0.75–1.97)	3 ↑ (0.4–2)	0.4 ↓ (0.75–1.97)
IgG g/l	5.80 (5–17)	19.03 ↑ (5–11)	16.38 ↑ (5–11)	9.034 (8–15)	15.6 (5–17)	16 ↑ (8–15)	14.6 (8–19)
IgA g/l	1.41 (0.85–2.2)	2.31 ↑ (0.55–1.2)	1.25 ↑ (0.55–1.2)	0.837 (0.75–2)	3.40 ↑ (0.85–2.2)	2.05 ↑ (0.75–2)	3.8 ↑ (0.85–2.2)
IgE IU/ml	18,730 ↑ (0–200)	7,174 ↑ (0–200)	3,029 ↑ (0–200)	16,534 ↑ (0–200)	99,600 ↑ (0–200)	141,300 ↑ (0–200)	9,320 ↑ (0–200)
<b>NBT</b>	normal	normal	normal	normal	normal	normal	normal

Arrows indicate values above (↑) or below (↓) the reference ranges. Normal ranges from 10<sup>th</sup> – 90<sup>th</sup> percentile. <sup>40</sup> WBC, white blood count; ANC, absolute neutrophil count; AEC, absolute erythrocyte counts; nd, not done; NBT, Nitro Blue Tetrazolium test.

TABLE III

## B and T cell phenotyping

(%)	B.V.6	B.V.7	C.IV.7	D.IV.2	D.IV.5	Travel control	Reference range
CD19 <sup>+</sup> B cells	12.26	28.11 ↑	1.07 ↓	9.04	7.06	18.6	4.9 – 18.4
CD27 <sup>+</sup> IgD <sup>+</sup> (naïve)	62.72	77.08	24.75 ↓	69.6	75.6	54.61	42.6 – 82.3 % CD19 <sup>+</sup>
CD27 <sup>+</sup> IgD <sup>+</sup> (IgM memory)	23.99	4.68 ↓	3.52 ↓	13.12	13.9	9.34	7.4 – 32.5 % CD19 <sup>+</sup>
CD27 <sup>+</sup> IgD <sup>-</sup> (switched memory)	7.70	7.30	52.39 ↑	12.66	5.59 ↓	21.62	6.5 – 29.1 % CD19 <sup>+</sup>
CD27 <sup>-</sup> IgD <sup>-</sup> (switched memory)	0.94	2.05	0.56	1.19	0.43	3.93	0.25–7.0 % PBL
CD21 <sup>-</sup> CD38 <sup>-</sup>	8.20 ↑	1.79	11.86 ↑	11.5 ↑	5.29	7.84	0.9 – 7.6 % CD19 <sup>+</sup>
Transitional B cells	2.34	0.81	8.89 ↑	4.92 ↑	7.92 ↑	1.48	0.6 – 3.4
Plasmablasts	0.05 ↓	0.05 ↓	14.82 ↑	3.45	1.3	0.09 ↓	0.4 – 3.6
CD3 <sup>+</sup> (total T cells)	78	58	88 ↑	95.7 ↑	91.9 ↑	56	52–83 % lymph
CD3 <sup>+</sup> CD4 <sup>+</sup> (CD4 T cells)	26	41	48	nd	nd	24	24–57 % lymph
CD3 <sup>+</sup> CD8 <sup>+</sup> (CD8 T cells)	45 ↑	14	32	nd	nd	24	9–39 % lymph
CD4 <sup>+</sup> CD5RO <sup>+</sup> (CD4 memory)	90 ↑	90 ↑	95 ↑	nd	nd	56	35–82 % CD4 <sup>+</sup>
CD4 <sup>+</sup> CD45RO <sup>-</sup> CXCR5 <sup>+</sup> (TFH)	5	8	2 ↓	nd	nd	2 ↓	4–13 % CD4 <sup>+</sup>
CD4 <sup>+</sup> CD45RO <sup>+</sup> CXCR5 <sup>+</sup> (TFH)	6	9	2 ↓	nd	nd	4	4–13 % memory
CD8 <sup>+</sup> CD27 <sup>-</sup> CD28 <sup>-</sup> (late effector)	71 ↑	25	84 ↑	48.9 ↑	58.1 ↑	10	2–36 % CD8 <sup>+</sup>
CD8 <sup>+</sup> CD27 <sup>+</sup> CD28 <sup>-</sup> (effector)	18 ↑	46 ↑	6	44.5 ↑	37.2 ↑	10	3–17 % CD8 <sup>+</sup>
CD4 <sup>+</sup> CD8 <sup>-</sup> (double negative T)	2	1	2	0.9	1.6	4 ↑	0.4–2.2 % TCRaB <sup>+</sup>
CD4 <sup>+</sup> CD45RA <sup>+</sup> (CD4 Naïve)	12 ↓	11 ↓	2 ↓	nd	nd	31	31–72 % CD4 <sup>+</sup>
CD4 <sup>+</sup> CD45RA <sup>+</sup> CD31 <sup>+</sup> (RTEs)	8 ↓	7 ↓	1 ↓	nd	nd	16	12–30 % CD4 <sup>+</sup>
CD4 <sup>+</sup> CD45RA <sup>+</sup> CD31 <sup>+</sup> (RTEs)	67	64	35 ↓	53.5	52.9	52	41–79 % naïve
CD4 <sup>+</sup> CD45RO <sup>+</sup> IFN $\gamma$ <sup>+</sup>	22.53	14.17	20.73	nd	nd	15.61	13.8–29 % CD4 <sup>+</sup> CD45RO <sup>+</sup>
CD4 <sup>+</sup> CD45RO <sup>+</sup> IL4 <sup>+</sup>	9.81	18.83 ↑	26.57 ↑	nd	nd	11.10	4–12.1 % CD4 <sup>+</sup> CD45RO <sup>+</sup>
CD4 <sup>+</sup> CD25 <sup>hi</sup> FoxP3 <sup>+</sup>	2.22	3.46	1.08 ↓	nd	nd	4.58	1.7–7.8 % CD4 <sup>+</sup>

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(%)	B.V.6	B.V.7	C.IV.7	D.IV.2	D.IV.5	Travel control	Reference range
CD4 <sup>+</sup> CD45RO <sup>+</sup> IL17 <sup>+</sup>	0.72 ↓	1.02 ↓	1.07 ↓	nd	nd	0.79 ↓	1.2-5.1 % CD4 <sup>+</sup> CD45RO <sup>+</sup>

TFH, follicular T-helper cells; RTEs, recent thymic emigrants; nd, not done.

**TABLE IV**Decreased *in vitro* T cell proliferation in patients with *PGM3* mutations.

Patient	PHA	anti-CD3	PPD	TT
A.V.12	35	29.1	5.6	4.1
A.V.13	30	2.5	1.2	1.9
A.V.14	24	6.4	4.8	1.9
A.V.18	25	12.8	9.6	1.7
B.V.6	186	45.3	2.0	2.3
B.V.7	71	51.4	9.3	1.4
control (n=4)	78±52.7	13±6	41±19.7	25±17

PBMCs were cultured in the presence of phytohemagglutinin (PHA) for 3 days or with anti-CD3 or recall antigens (purified protein derivative, PPD; tetanus toxoid, TT) for 5–6 days. Values are expressed as stimulation indexes (SI) calculated as mean counts per minute of triplicates in stimulated cultures versus controls. For the controls, standard deviation of the mean is indicated. SI<2 are considered absent.

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