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Autosomal recessive *PGM3* mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive impairment

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Abstract

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Background—Identifying genetic syndromes that lead to significant atopic disease can open new pathways for investigation and intervention in allergy.

Objective—To define a genetic syndrome of severe atopy, elevated serum IgE, immune deficiency, autoimmunity, and motor and neurocognitive impairment.

Methods—Eight patients from two families who had similar syndromic features were studied. Thorough clinical evaluations, including brain MRI and sensory evoked potentials, were performed. Peripheral lymphocyte flow cytometry, antibody responses, and T cell cytokine production were measured. Whole exome sequencing was performed to identify disease-causing mutations. Immunoblotting, qRT-PCR, enzymatic assays, nucleotide sugar and sugar phosphate analyses along with MALDI-TOF mass spectrometry of glycans were used to determine the molecular consequences of the mutations.

Results—Marked atopy and autoimmunity were associated with increased T_H2 and T_H17 cytokine production by $CD4^+$ T cells. Bacterial and viral infection susceptibility were noted along with T cell lymphopenia, particularly of $CD8^+$ T cells, and reduced memory B cells. Apparent brain hypomyelination resulted in markedly delayed evoked potentials and likely contributed to neurological abnormalities. Disease segregated with novel autosomal recessive mutations in a single gene, phosphoglucomutase 3 (*PGM3*). Although PGM3 protein expression was variably diminished, impaired function was demonstrated by decreased enzyme activity and reduced UDP-GlcNAc, along with decreased O- and N-linked protein glycosylation in patients' cells. These results define a new Congenital Disorder of Glycosylation.

Conclusions—Autosomal recessive, hypomorphic *PGM3* mutations underlie a disorder of severe atopy, immune deficiency, autoimmunity, intellectual disability and hypomyelination.

Keywords

Atopy; *Immune deficiency*; *Hyper-IgE*; *neurocognitive impairment*; *phosphoglucomutase 3*; *glycosylation*; *Allergy*; *Autoimmunity*

INTRODUCTION

Mendelian atopic diseases provide opportunities to discover new pathophysiologic mechanisms contributing to allergy. Often, such diseases are identified because of features besides atopy, such as immunodeficiency (Hyper IgE syndrome, DOCK8 deficiency, Omenn's syndrome, Wiskott-Aldrich syndrome, ADA-SCID), autoimmunity (IPEX syndrome), and non-immune abnormalities (developmental delay and abnormal skin in prolidase deficiency). When they co-segregate with atopy, these associated features help characterize the disease as a familial disorder, and demonstrate the full phenotypic spectrum caused by the underlying molecular lesion(s).

The prototype of such monogenic disorders is the autosomal dominant Hyper IgE syndrome (HIES), which was initially described because of recurrent staphylococcal infections [1], but was later found to be associated with elevated IgE [2]. The identification of autosomal dominant *STAT3* mutations in HIES established a role for this transcription factor in marked IgE elevation [3, 4], and more recently in protection from mast cell degranulation [5]. By

contrast, autosomal recessive *DOCK8* mutations lead to viral skin infections, mucocutaneous candidiasis, and severe atopic disease including eczema, asthma, food allergies, and anaphylaxis [6–8]. Such patients have increased T_H2 cells (IL-4, IL-13), pointing to a role for DOCK8 in T cell regulation of allergic responses [9]. Although *STAT3* and *DOCK8* mutations account for many cases of marked IgE elevation, the majority of patients with increased serum IgE and atopic disease in addition to syndromic features still have no identified genetic cause. These include an unusual kindred previously described at our center, which had recurrent infections, cutaneous vasculitis, motor and neurocognitive impairment, and other non-immune abnormalities [10].

Diseases that impact multiple organ systems, such as the one in the kindred mentioned above, include Congenital Disorders of Glycosylation (CDG). Typical features of CDG are extremely broad, but can include motor and neurologic deficits, hematologic abnormalities, dysmorphism, and other malformations. Abnormal immune function has been observed, including hypogammaglobulinemia with decreased B cell numbers in ALG12-CDG (also called CDG-Ig) due to mutations in *ALG12*[11], leukocyte adhesion deficiency type 2 or SLC35C1-CDG due to mutations in *SLC35C1* (also called CDG-IIc) [12], glucosidase I deficiency MOGS-CDG or CDG-IIb[13]. The widespread clinical manifestations are thought to be due to the ubiquity of glycosylation and its central roles in an array of normal cellular functions. During glycosylation, sugar chains are added to either proteins or lipids, using basic sugar building blocks such as UDP-N- acetyl-glucosamine (UDP-GlcNAc). After being generated through the hexosamine biosynthetic pathway or through the salvage pathway, UDP-GlcNAc is used to make N- glycans, O-glycans, proteoglycans, and glycosylphosphatidylinositol (GPI)-anchored proteins within the cell. These glycosylated proteins are found in various cellular compartments, on the cell surface, or in the plasma and extracellular matrix. Additionally, UDP-GlcNAc is also used for O-GlcNAc addition in the cytosol or nucleus, where it participates in cell signaling[14].

Here we report the discovery of a genetic defect in glycosylation precursor synthesis causing a novel disease in eight patients from two families. The patients have severe atopy with marked serum IgE elevations, recurrent bacterial and viral infections, and motor and neurocognitive impairment most likely associated with hypomyelination. Their mutations, which affect an enzyme crucial in the generation of UDP-GlcNAc, point to a previously unappreciated role for glycosylation in the regulation of atopic disease, as well as associated comorbidities. Our findings suggest that altered glycosylation may be important in the pathophysiology of allergic diseases in the general population.

METHODS

Subjects

Patients and their families provided informed consent on NIH IRB-approved research protocols designed to study atopy (NCT01164241), hyper-IgE syndromes (NCT00006150), general host defense defects (NCT00001355), and/or lymphocyte homeostasis disorders (NCT00246857). Comprehensive histories, review of all available outside records, serial clinical evaluations, and therapeutic interventions were all performed at the Clinical Center of the National Institutes of Health (NIH). Clinical immunologic laboratory tests were

performed by the Department of Laboratory Medicine at NIH, Bethesda, MD. Glycan profile quantitation and analysis in blood and urine were performed using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectroscopy by Emory Genetics Laboratory, Decatur, GA.

Detailed procedures and additional information on genetic analysis, PCR and DNA sequencing, immunoblot analysis, structural analysis, enzyme activity assay, sugar phosphate and nucleotide sugar analysis, flow cytometric analysis, and MRI are provided in the Methods section in this article's Online Repository.

RESULTS

Clinical phenotype of individuals with hypomorphic *PGM3* mutations

Much of the clinical phenotype of this syndrome was first reported in Family I as an autosomal recessive immunodeficiency-vasculitis-myoclonus syndrome [10]. A second family comprising three male children of two consanguineous couples from Egypt was more recently identified with many phenotypic similarities (Figure 1). Both families were initially referred for evaluation because of atopic dermatitis, recurrent skin and pulmonary infections, and high serum IgE; *DOCK8* and *STAT3* sequences were found to be wild type.

All affected individuals had atopic dermatitis that was frequently severe, as well as atopic diatheses including asthma, food, drug, and environmental allergies, and elevated serum IgE. Two individuals from Family I (I.1 and I.3) required repeated inpatient wet wrap therapy with topical corticosteroids and emollients; both individuals displayed a marked response to therapy, consistent with that seen in other patients with severe atopic dermatitis (Figure 2A). Those in Family I also developed severe blistering skin disease as toddlers, on the spectrum of Stevens-Johnson syndrome. Because they occurred without identifiable exogenous provocation, we refer to them as erythema multiforme major (Table I). Skin infections were prominent, with recurrent staphylococcal soft tissue infections (SSTI) in all patients, molluscum contagiosum in patient I.3, and flat warts in patient II.1 (Table I). Mild defects in T cell function were also suggested by persistent low-level EBV viremia despite detectable EBV IgG in the older living patients in Family I. This was associated with the development of EBV^+ Nodular Sclerosing Hodgkin's Lymphomas (NSHL) in identical twin patients I.3 and I.4, which were successfully treated with Adriamycin / Bleomycin / Vinblastine / Dacarbazine (ABVD) chemotherapy (Table I).

Patients had recurrent pneumonias (n = 6), recurrent acute otitis media (n = 3), chronic otitis externa (n = 6), and chronic sinopulmonary disease (n = 6) (Table I). Most pneumonias were presumed bacterial in origin, although patient II.1 developed a fungal pneumonia after receiving high dose inhaled corticosteroids (Table I). The recurrent infections occurred in spite of elevated serum immunoglobulins and protective antibody titers (Table II). Anti-microbial prophylaxis proved moderately beneficial, but immunoglobulin replacement trials failed to reduce these infections in a clinically significant way. Moderate to severe bronchiectasis developed among older patients, leading to chronic respiratory failure, death of patient I.5 at age 27 years, and bilateral lung transplantation in patient I.3 at age 28 years.

Autoimmune and idiopathic immune-mediated disease was also prevalent. Cutaneous leukocytoclastic vasculitis was present in all surviving patients in Family I, as previously reported. Membranoproliferative glomerulonephritis developed in two of these patients (Table I); one of whom progressed to end-stage renal disease (ESRD) at age 30 years and remains on hemodialysis (HD) after failing deceased-donor renal transplantation (DDRT). Severe autoimmune/idiopathic neutropenia requiring G-CSF (patient II.1), and hemolytic anemia (patient II.2) with osmotic fragility also occurred.

Finally, neurologic impairment was evident from early in life in all patients. Features included developmental delay (n = 7), low IQ (n = 7), ataxia (n = 7), dysarthria (n = 5), sensorineural hearing loss (n = 4), myoclonus (n = 5), and seizures (n = 1). Facial dysmorphism was appreciated in several patients. Baseline electroencephalography (EEG) showed moderate background slowing, poor organization, and a slowed posterior dominant rhythm in two patients. In one patient, voluntary movement induced sharp waves and slowing in the contralateral motor cortex seen on scalp EEG, which were consistent with cortical intention myoclonus[15]. The neurologic impairment appeared to be associated with diminished myelination, as T2-FLAIR MRI showed stable diffuse hyperintensity on images within the central white matter (n = 4 of 6 patients scanned) (Figure 2B); these findings were stable over time. Furthermore, visual evoked potentials (VEPs) showed prolonged p100 latencies in both eyes (OS 200.2 ms and OD 197.1 ms) at check size 16 (normal 84 -120 ms) (n = 2) (Figure 2B). Somatosensory evoked potentials (SSEPs) also showed delayed central conduction in the same individuals, after stimulation of bilateral upper extremities as well as "giant" SSEPs (peak-to-peak amplitudes of the N20-P22 waveforms approximately 18.7 μV left and 15 μV right). Of note, patient II.1 developed *Streptococcus pneumoniae* meningoencephalitis; perivenular white matter lesions were seen on MRI following this episode.

Clinical laboratory findings

Patients had significant leukopenia, lymphopenia, and neutropenia, especially in Family II (Table II). Further immunophenotyping showed the lymphopenia to be predominantly due to low $CD8^+$ T cells and $CD27^+$ memory B cells (Table II). Patients had significant elevations in serum IgE, IgG, and IgA (Table II), and protective antibodies to protein and polysaccharide antigens. Of the autoantibodies screened, only rheumatoid factor (RF) was consistently present, occurring in five of seven patients tested (Table II).

Genetic analyses

Because patients of both sexes in Family I were affected but their parents were not, we assumed an autosomal recessive model in searching for causal genetic variants. Whole exome sequencing produced about 80–110 million paired-end reads per exome and provided an average 80–100 fold sequencing coverage of >90% protein-coding regions. About 70,000 high confidence single nucleotide variants (SNVs) and small insertions/deletions (indels) were called from individually sequenced exomes. Variants were flagged if they met the following criteria: 1) they were nonsense, missense, splice site mutations/indels, or frameshift indels, which altered protein-coding sequence; 2) they were novel or rare when compared to several public variation databases including the dbSNP database and the

NHLBI exome variant Server; and 3) they were shared by all 4 sequenced patients in the same family. Any gene that was identified to carry two or more such variants was further investigated by filtering against the genotype of those variants in the parents. After this step, *PGM3* (Phosphoglucomutase 3, NM_001199917.1, OMIM 172100) emerged as the only possible candidate gene. We identified two novel compound heterozygous mutations in Family 1's *PGM3*, which completely segregated with disease: a missense variant (c. 1585G>C, p. E529Q) was inherited from the mother; a 5 bp deletion (c.1438_1442del , p. L480Sfs*10), causing a frameshift and premature stop was inherited from the father (Figure 3A). *PGM3* genotyping was confirmed by Sanger sequencing.

In Family II, WES was initially performed on patients II.1 and II.2. We used a similar strategy as in Family I for filtering, except that homozygous variants were favored because the parents were consanguineous. After filtering, only 15 rare/novel variants compatible with all the criteria remained. One of these was a missense mutation in *PGM3* (c.975T>G, p.D325E, Figure 3A). Sanger sequencing of *PGM3* in an affected cousin identified the same homozygous mutation as well as heterozygous carrier status in that patient's unaffected parents.

Expression of PGM3 variants

PGM3 is a member of the hexose phosphate mutase family and catalyzes the reversible conversion of GlcNAc-6-phosphate (GlcNAc-6-P) to GlcNAc-1-P. This is a key step in the synthesis of UDP-GlcNAc, which is critical for multiple glycosylation pathways including N- and O-linked. The structure of *PGM3* and the locations of the mutations in the gene are shown in Figure 3B. Both missense mutations in the patients result in the substitution of highly conserved amino acids (D325E and E529Q, Figure 3C). By analogy to the structure of its closest available homolog, yeast N-acetylglucosamine- phosphate mutase, both missense mutations are proximal to a central active site cavity that is surrounded by active serine, metal binding, sugar binding, and phosphate binding domains (domains 1 to 4, respectively) (Figure 3D). E529 is in the conserved substrate- binding loop of PGM3. Replacement of glutamine with glutamate removes a negatively charged moiety that normally interacts with positively charged K481; disruption of this interaction likely destabilizes a defined substrate-binding conformation within the loop. By contrast, D325 is located at the interface between the metal binding and sugar binding domains, where it is expected to neutralize its net negative charge by interacting with positively charged K328. Replacement of aspartic acid with glutamic acid increases the length of the side-chain, which likely forces rotation of the side-chain into the interdomain region; this would break several critical hydrogen bonds and perturb interdomain regions near the active site that are critical for catalysis. Thus, both missense mutations are expected to be hypomorphic.

To assess whether the mutations affected PGM3 protein levels, we performed immunoblotting using lysates from EBV-transformed B cell lines or cycling T cells derived from patients and controls. PGM3 protein levels were significantly decreased in patients from Family II but were not changed or only slightly decreased in Family I (Figure 3E). qRT-PCR showed normal *PGM3* mRNA expression in cells derived from Family II (Figure E1), which further argued for a protein-destabilizing effect of the D325E variant. For Family

I, the 5 bp deletion is predicted to result in a truncated protein (L480fs), but no such truncated protein band was detected (Figure 3E). This mutation is also predicted to cause nonsense-mediated decay of transcript, which likely contributes to the mild diminution of PGM3 protein expression (Figure 3E). The mild decrease in protein expression in Family I also suggests that the E529Q protein is expressed and has normal protein stability.

Hypomorphic *PGM3* mutations encode proteins with reduced enzymatic activity

To assess the effects of *PGM3* mutations, we developed a novel enzymatic assay and tested fibroblasts from three patients by determining the rate of GlcNAc1-P formation from GlcNAc6-P. All patients had substantially decreased PGM3 activity compared to control cells (Figure 4A). As this is an easily reversible reaction, we measured the intracellular amounts of GlcNAc1-P and GlcNAc6-P. Patient fibroblasts had no change in GlcNAc1-P but a significant increase in the amount of GlcNAc6-P (Figure 4B). Conversion of GlcNAc6-P to GlcNAc1-P is a key step in the synthesis of UDP-GlcNAc; thus, we also measured the amount of UDP-GlcNAc and other nucleotide sugars in cell extracts. PGM3-deficient cells showed significant reduction of UDP-GlcNAc and somewhat reduced level of UDP-GalNAc, which is derived from UDP-GlcNAc, but other nucleotide sugars remained normal (Figure 4C). Importantly, GlcNAc supplements in the medium restored depleted UDP-GlcNAc and UDP-GalNAc compared to controls (Figure 4C).

Abnormal glycosylation in patients with hypomorphic *PGM3* mutations

O-linked glycosylation in 5 patients (2 from Family I, and all affected individuals in Family II) was studied by mass spectrometric analysis of single samples. All individuals showed increased T antigen (Galβ1,3GalNAc), a precursor of sialylated T antigen (Sia2,3 Galβ1,3GalNAc). (Table III). The high T-antigen/Sialyl T-antigen ratio suggests hypo-sialylation, and is consistent with decreased UDP-GlcNAc, a precursor to CMP- Sialic acid. Serum transferrin glycosylation was normal, but analysis of total N-linked glycans showed under-galactosylation of N-linked oligosaccharides in 3 individuals.

Immune abnormalities in the patients

We next assessed various T cell functions including cytokine production and proliferation. *Ex vivo* stimulation of $CD4^+$ T cells from patients led to a marked increase in T_H2 cytokine production compared to normal controls, as indicated by elevated levels of IL-4, IL-5 (Figure 4D and Figure E2), and IL-13 (data not shown). IL-17 production was increased in both families, especially in Family II. By contrast, IFN-γ expression in patients was not significantly different from controls (Figure 4D). While the patients had fewer T cells than controls, measurement of CFSE dilution in SEB-stimulated total PBMCs led to similar or increased proliferation of gated $CD4^+$ and $CD8^+$ cells in patients as compared to normal controls. Similar results were observed upon stimulation of PBMCs by the lectin PHA-P (Figure E3).

DISCUSSION

Whole exome sequencing has proven useful for the unbiased identification of mutations in genes whose functions are not limited to the immune system. We have now discovered a

new syndrome of severe atopy, recurrent infections, autoimmunity, vasculitis, renal failure, and lymphoma, associated with motor and neurocognitive impairment. These patients have novel recessive mutations in phosphoglucomutase 3 (*PGM3*) and show hypo-sialylation of O-linked serum glycans, consistent with impaired PGM3 function. In conjunction with the nine patients identified by Barbouche *et al* (see accompanying article), a total of 17 patients from six families have now been identified, making a compelling argument for the pathogenicity of these mutations.

PGM3 is required for glycosylation through catalyzing the isomerization of N-acetyl-glucosamine-6-phosphate to N-acetyl-glucosamine-1-phosphate during the generation of UDP-GlcNAc. Consistent with this role, inactivating mutations in the *Drosophila* homolog *nst* cause severe defects in mesodermal development [16]. Complete loss of *Pgm3* is embryonically lethal in mice, while hypomorphic mutations in *Pgm3* allow viability [17]. Our patients had partial loss of PGM3 function, which allowed them to survive into adulthood, but they still had a distinct phenotype that recapitulated that seen in hypomorphic mice. Both humans and mice had lymphopenia with $CD8^+$ and B cell defects, autoimmunity such as glomerulonephritis, and other hematologic abnormalities. Other phenotypes not originally examined such as atopic disease, high serum IgE, and neurological abnormalities, may well be present in the mouse.

Whereas PGM3 deficiency presented to us with elevated IgE, atopic dermatitis, bronchiectasis, and scoliosis, other clinical features helped to distinguish this disorder from the two best-known diseases with elevated IgE, *STAT3* and *DOCK8* deficiencies (Table IV). PGM3 deficiency is unassociated with cold abscesses, chronic mucocutaneous candidiasis, retained childhood dentition, and joint hypermobility, which are seen very commonly in patients with *STAT3* mutations. Although PGM3-deficient patients occasionally have viral skin infections, they were not as prevalent as in DOCK8 deficiency. By contrast, primary neurocognitive deficits and probable hypomyelination, suggested by the evoked potential findings and consistent with the MRI scans, are seen in PGM3 deficiency but not in either STAT3 or DOCK8 deficiencies. The neurologic features are evocative of other glycosylation defects, such as DPAGT1-CDG (CDG Ij) and MOGS-CDG (CDG-IIb), which are both associated with primary hypomyelination. These overlaps in three distinct glycosylation disorders indicate an important role for glycosylation in normal axonal health [13].

In our patients, we also observed abnormal effector cytokine production, which might contribute to their inflammatory and autoimmune symptoms. PGM3-deficient T cells produced excessive T_H17 and T_H2 cytokines while maintaining normal IFN-γ production (Figure 4D). While excessive T_H2 cytokine production may explain the atopic disease and hypergammaglobulinemia, it is unclear whether it contributes to the vasculitis or autoimmune kidney disease. The excessive IL-17 production can be associated with demyelinating diseases, but the diffuse and stable hypomyelination observed in these patients argues against an inflammatory etiology. We have not yet been able to establish an immunologic mechanism to explain the link between glycosylation abnormalities and the immune dysregulation seen in these patients. Because glycosylation is known to be critical for a large number of immune-related proteins, these patients likely have additional

abnormalities in numerous other pathways affecting leukocyte development and maturation. Such abnormalities will need to be examined in order to establish the precise mechanism by which these mutations and abnormal glycosylation lead to focal cellular and clinical defects in immune responses. Analysis of these pathways should illuminate how differential protein glycosylation contributes to the basic functioning of many primary immunologic and non-immunologic pathways, including those that lead to severe atopic phenotypes.

Finally, we found that GlcNAc supplementation restored intracellular UDP- GlcNAc levels in PGM3-deficient cells. Thus, exogenous non-diabetogenic sugars might be used to bypass the metabolic defect and treat these patients. A similar therapeutic approach has been taken using oral mannose in patients with MPI-CDG (CDG-Ib), due to mutation in mannosephosphate isomerase [18]. Identifying a role for glycosylation events in patients with atopic disease opens new avenues for pharmacological manipulation to treat common allergic diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ABVD	Adriamycin / Bleomycin / Vinblastine / Dacarbazine
ADA-SCID	Adenosine deaminase - severe combined immunodeficiency
CDG	congenital disorder of glycosylation
dbSNP	The Single Nucleotide Polymorphism Database
DDRT	deceased-donor renal transplantation
DOCK8	dedicator of cytokinesis 8
EBV	Epstein-Barr virus
EEG	electroencephalography
ESRD	end-stage renal disease

GlcNAc	N-acetyl-D-glucosamine
GlcNAc-6-P	GlcNAc-6-phosphate
GlcNAc-1-P	GlcNAc-1-phosphate
GPI	glycosylphosphatidylinositol
HD	hemodialysis
HIES	Hyper-IgE syndrome
IPEX	Immune Dysregulation Polyendocrinopathy Enteropathy X-linked
MALDI-TOF MS	Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry
NSHL	Nodular Sclerosing Hodgkin's Lymphomas
PBMC	Peripheral blood mononuclear cel
PGM3	phosphoglucomutase 3
PHA	phytohemagglutinin
PMA	phorbol 13-myristate 13-acetate
qRT-PCR	quantitative reverse transcription polymerase chain reaction
SEB	Staphylococcal enterotoxin B
SNV	Single Nucleotide Variant
SSEP	Somatosensory evoked potentials
SSTI	staphylococcal soft tissue infections
STAT3	signal transducer and activator of transcription 3
TH2	T helper type 2 cells
TH17	T helper type 17 cells
UDP-GalNAc	Uridine diphosphate N-acetylgalactosamine
UDP-GlcNAc	Uridine diphosphate N-acetylglucosamine
VEP	visual evoked potentials
WES	whole-exome sequencing

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Clinical implications

Our findings define a new clinical entity of atopy, immune deficiency, autoimmunity with neurocognitive impairment in patients associated with diminished PGM3 function.

Capsule summary

This study reports a novel genetic defect leading to a syndrome that includes significant atopic disease and indicates an important role for glycosylated proteins in the pathogenesis of allergic disease.

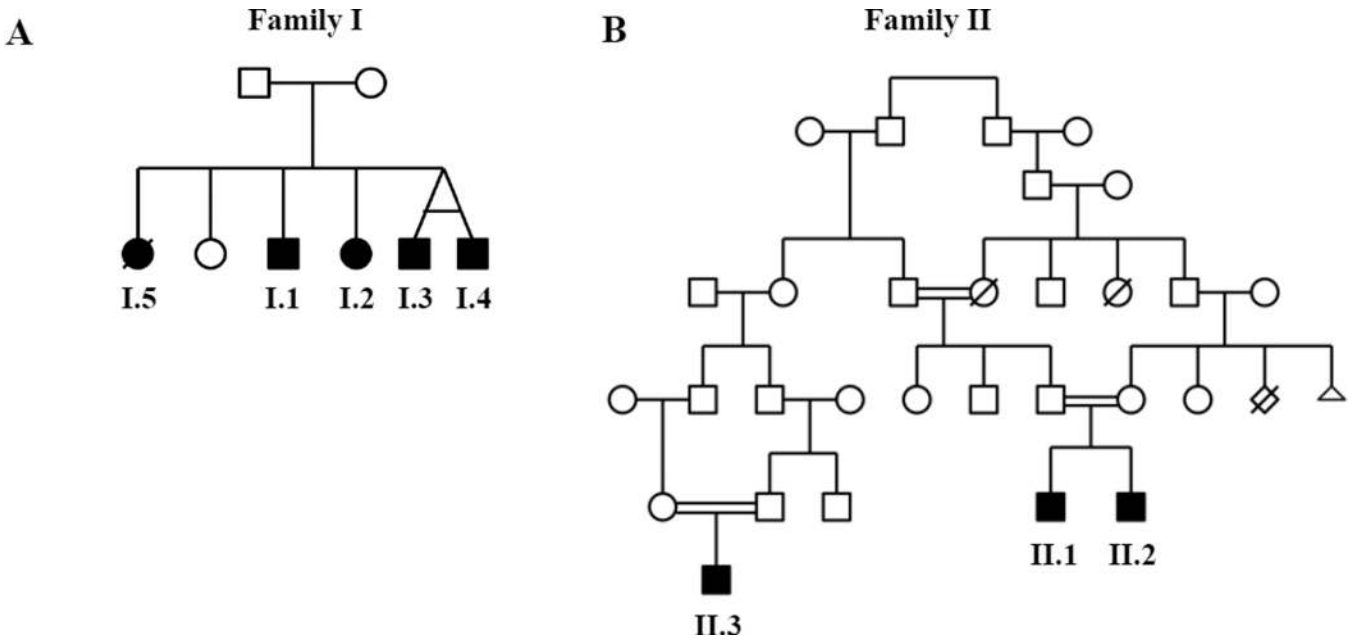


Figure 1. Pedigrees of the two families in the study

Solid symbols denote affected status and a slash through a symbol represents a deceased person.

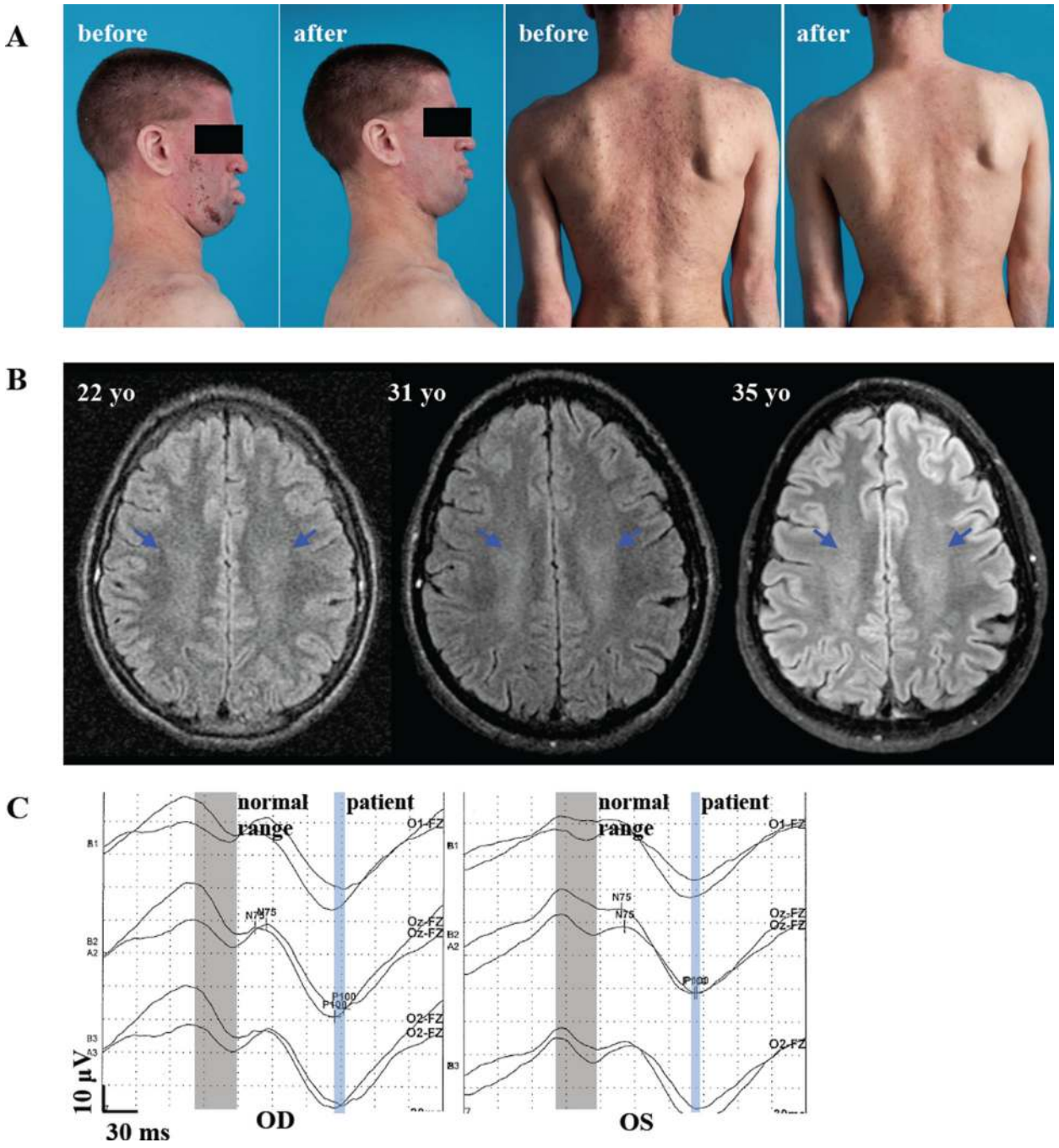


Figure 2. Clinical findings in patients

A, Severe atopic dermatitis in Patient I.1, before and after wet wrap therapy with topical corticosteroids and emollients. Note scoliosis. B, Brain MRI, axial T2-FLAIR images of Patient I.2 showing stable hyperintensity in the white matter semiovale (blue arrows) at ages 22 (left), 31 (middle) and 35 (right) years, suggestive of stable dysmyelination. These findings were typical across our cohort. C, Visual evoked potentials in Patient II.1 demonstrate a prolonged p100 in both eyes (OS 200 ms and OD 197 ms) at check size 16,

blue arrows, consistent with demyelinating optic neuropathy; x-axis 30ms per box, y-axis 10μV per box; normal p100 peak latency indicated by grayed areas (84.1–119.5 ms).

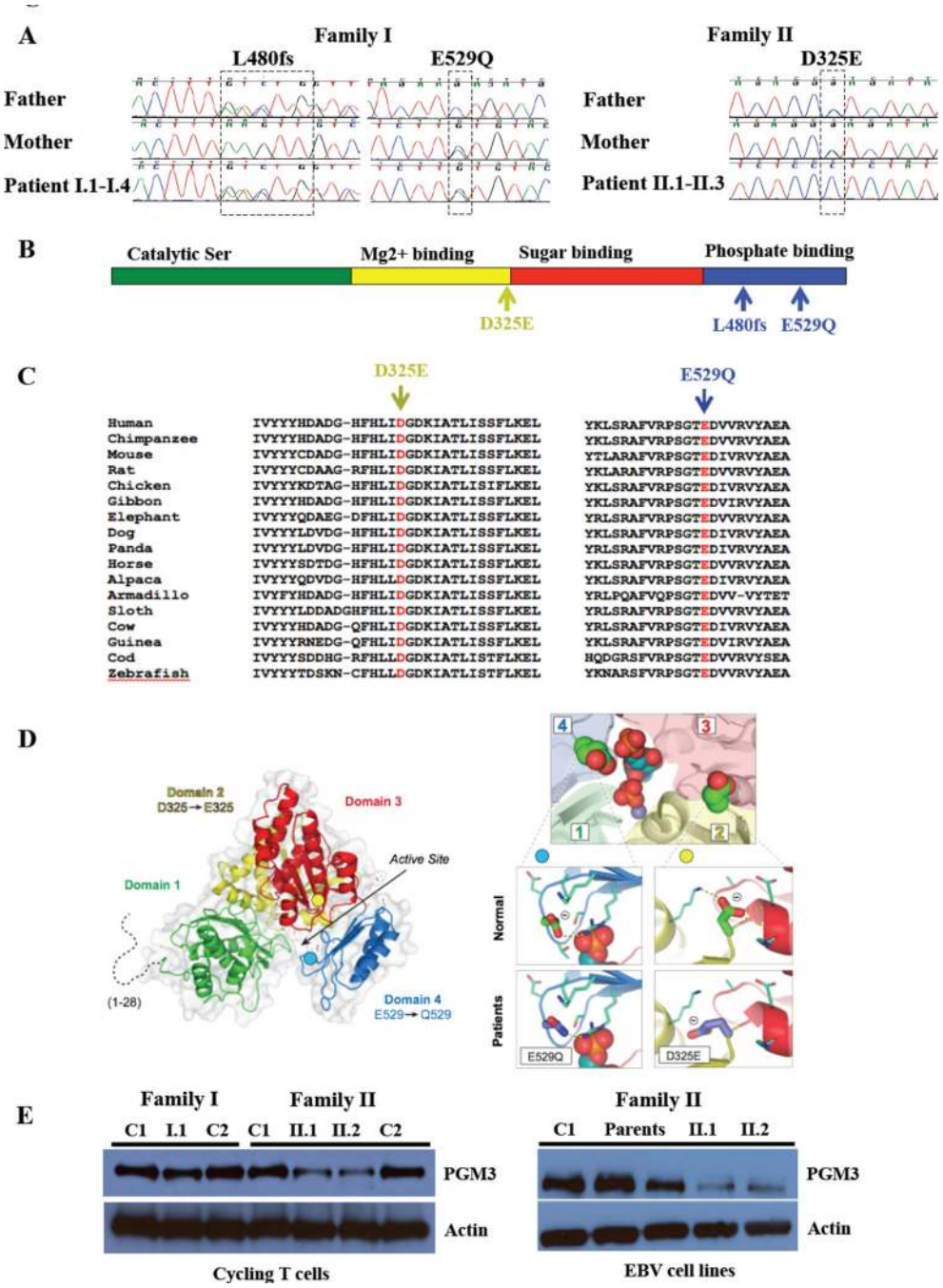


Figure 3. Genetic analysis of PGM3 deficiency

A. Compound heterozygous *PGM3* mutations in Family I, and homozygous missense *PGM3* mutation in Family II

B. PGM3 domain structure. The mutations detected in Family I (blue) and Family II (yellow) are indicated by arrowheads.

C, Multiple sequence alignment of PGM3 from different species, showing conservation of residues that are mutated in the patients.

D, Structural model of PGM3. Left panel, three-dimensional structural model of PGM3 protein, based on the structure of the closest available homolog from *Candida albicans*. Relative locations of the patient missense mutations are denoted as circles (D325 in yellow, E529 in blue). Right panel, positions of both mutations relative to the active site and bound substrate. Hydrogen bonds between the focal amino acid and any adjacent residues are shown as yellow dashes. The charge of the side chain is denoted, if applicable.
E, PGM3 protein levels in cell lysates from cycling T cells (left) and EBV cell lines (right), prepared from patients of both families, parents of Family II, and healthy control subjects. β-actin levels were measured as a loading control.

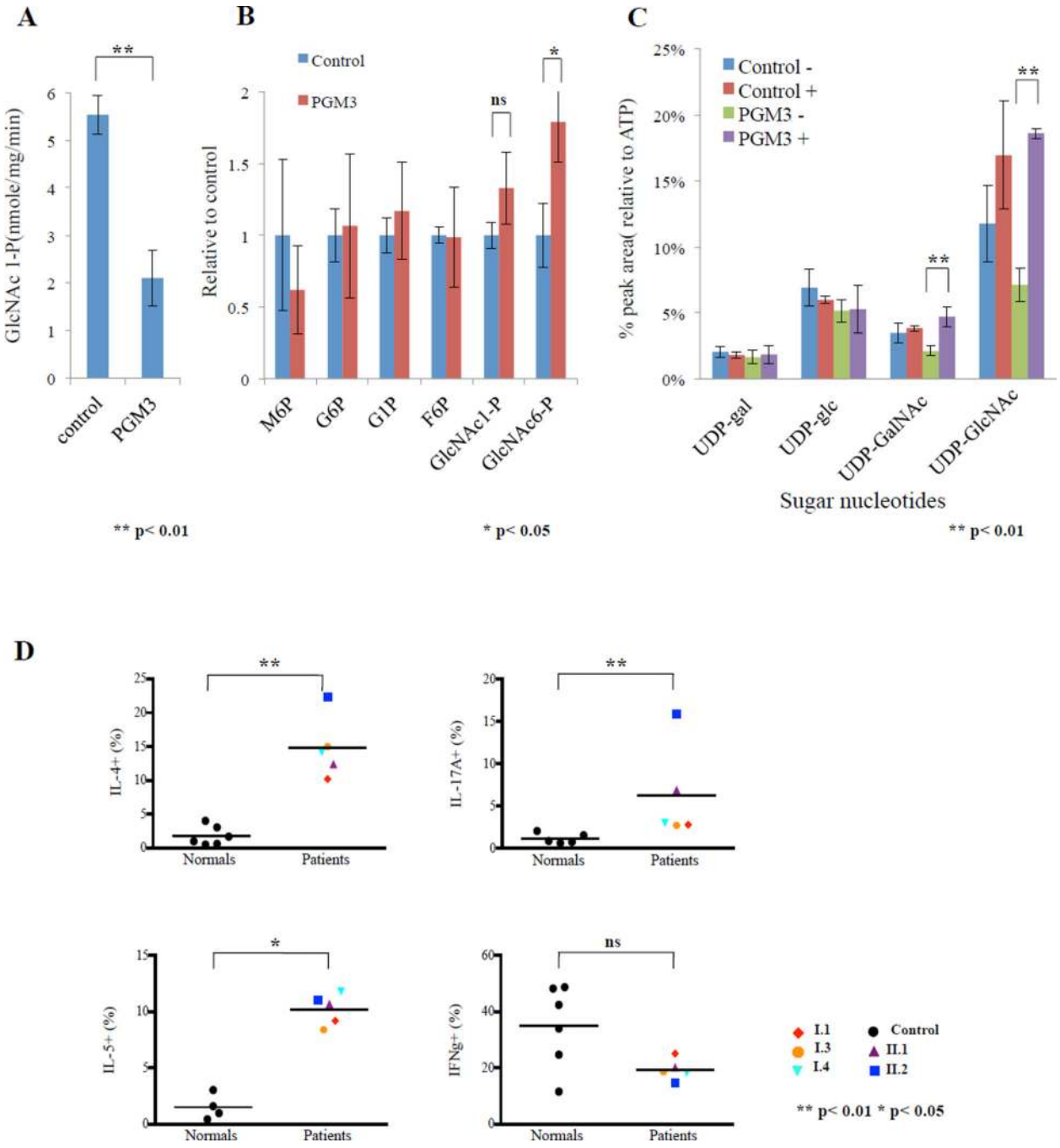


Figure 4. Impaired functions in PGM3-deficient patients

A, PGM3 enzymatic activities. The rate of GlcNAc1-P formation from GlcNAc6-P was measured in fibroblast lysates from patients (n=3) or normal healthy controls (n=2).

B, Sugar phosphate analysis, quantitated on samples from A.

C. Nucleotide sugar phosphate analysis, after overnight treatment of fibroblasts (three patients with two normal controls) with (+) or without (-) 10 mM GlcNAc. Exogenous GlcNAc supplementation increased UDP-GlcNAc and UDP-GalNAc levels.

D. Elevated T_H2 and T_H17 cytokines in patients with *PGM3* mutations. Percentage of $CD3^+$ $CD4^+$ $CD45RO^+$ cells producing IL-4, IL-5, IL-17 or IFN-γ in response to PMA and ionomycin (PMA/I) in PBMCs from normals and patients. Horizontal bars represent mean percentage for each of the groups. Experiments were performed 2–4 times for each patient.

Table I

Clinical characteristics of individuals with hypomorphic *PGM3* mutations.

	Pt.	Age/Sex	Allergic	Dermatologic	Infectious	Neurologic	Connective Tissue	Pulmonary	Renal	Hematologic
Family I	I.1	35yo M	allergic rhinitis	atopic dermatitis, vasculitis, erythema multiforme major	pneumonia, otitis, SSTI	low IQ, dysarthria, ataxia, myoclonus, sensorineural hearing loss, CNS myelination defect, abnormal EEG, prolonged VEP	scoliosis, degenerative disc disease, dilated aortic root	bronchiectasis	MPGN on hemodialysis	polycythemia
	I.2	32yo F	food allergy, allergic rhinitis, drug allergy	atopic dermatitis, vasculitis, erythema multiforme major	EBV viremia, sinopulmonary	low IQ, dysarthria, ataxia, myoclonus, sensorineural hearing loss, CNS myelination defect, seizure, abnl EEG	esophageal stricture	bronchiectasis s/p bilateral lung transplantation	lithiasis	
	I.3	30yo M	asthma, food allergy	atopic dermatitis, vasculitis, erythema multiforme major	EBV viremia, pneumonia, molluscum, otitis, SSTI	low IQ, dysarthria, ataxia, myoclonus, reduced vibratory sensation, sensorineural hearing loss	scoliosis, esophageal diverticulis	bronchiectasis		Hodgkin's lymphoma (EBV+)
	I.4	30yo M	food allergy, drug allergy	atopic dermatitis, vasculitis, erythema multiforme major	EBV viremia, otitis, SSTI	low IQ, dysarthria, ataxia, myoclonus, sensorineural hearing loss	scoliosis	bronchiectasis	MPGN	Hodgkin's lymphoma (EBV+)
	I.5	deceased (27yo F)	asthma, allergic rhinitis	atopic dermatitis, eczema herpeticum, erythema multiforme major	HSV esophagitis, otitis, sinopulmonary	ataxia, myoclonus	esophageal stricture	bronchiectasis, chronic failure		
Family II	II.1	12yo M	food allergy, allergic rhinitis, drug allergy	atopic dermatitis	SSTI, fungal pneumonia, otitis, sinopulmonary	low IQ, developmental delay, ataxia, CNS myelination defect, meningoencephalopathy, febrile seizure, prolonged VEP				neutropenia (+anti-neutrophil Ab)
	II.2	10yo M	allergic rhinitis	atopic dermatitis	flat warts, SSTI, otitis	low IQ, developmental delay, ataxia, CNS myelination defect, autism, febrile seizure	microcephaly, scoliosis		unilateral agenesis	hemolytic anemia, hepatosplenomegaly
	II.3	1yo M	food allergy, FPIES	atopic dermatitis	RSV, SSTI, otitis	developmental delay, hypotonia				neutropenia

FPIES, food protein-induced enterocolitis syndrome; EBV, Epstein-Barr virus; SSTI, recurrent staphylococcal skin and soft tissue infections; sinopulmonary, recurrent sinopulmonary infections; otitis, chronic otitis externa and/or recurrent otitis media; HSV, herpes simplex virus; RSV, respiratory syncytial virus; VEP, visual evoked potentials; MPGN, membranoproliferative glomerulonephritis.

Table II

Significant immune laboratory findings of individuals with hypomorphic *PGM3* mutations.

		Immunoglobulins				CBC				CD3			CD20		NK	EBV	RF
	Patient	**IgE IU/mL (0–90)**	**IgG mg/dL (759–1,550)**	**IgA mg/dL (58–359)**	**IgM mg/dL (35–239)**	**WBC 10^3 cells/ µL**	**ANC 10^3cells/ µL**	**ALC 10^3cells/ µL**	**AEC 10^3cells/ µL**	**CD4 cells/µL (359–1,565)**	**CD8 cells/µL (178–853)**	**CD4/CD 8 ratio**	**Total cells/µL (61–321)**	**$CD27^+$ Memory cells/µL (12–68)**	**cells/µL (126–729)**	**10^3 copies/ mL**	**(<15 IU/mL)**
Family I	I.1	11,200	1610–1,770	284–547	41–74	3.1–5.3	2.1–3.3	0.6–0.9	0.1–0.5	128	129	1	253	7	113	5.0	POS
	I.2	5,836–27,200	1,510–1,890	749–1,660	125–176	4.8–9.2	2.9–7.1	0.6–1.1	0.2–0.5	195–487	70–137	2.8–3.6	31–161	7	202–270	30.0	POS
	I.3	4,672–35,400	422–1,750	515–1,140	121–411	1.7–5.5	0.9–4.4	0.2–0.8	0.0–1.1	93–151	11–19	8–11	100–123**	2–3**	15–62	2.2	POS
	I.4	23,594–35,445	673–1,470	509–877	103–130	2.7–4.7	1.4–3.5	0.3–0.7	0.0–0.9	150–248	22–43	5.8–6.8	37–140**	4–10**	30–131	28.2	POS
	I.5	ND	ND	ND	ND	11.4–19.3	11–12.0	0.6–11.4	0–3.6	ND	ND	ND	ND	ND	ND	ND	ND
Family II	II.1	1,244–30,766	1,080–1,690	334–497	86–147	1.3–5.4	0.1–3.3*	0.7–1.3	0–0.4	368–692 §	111–247§	2.8–5.1§	16–108§	1§	150–260§	NEG	NEG
	II.2	5,396–11,405	1,660–2,180	407–677	42–144	1.0–3.5	0.4–1.7	0.4–2.0	0–0.2	403–878	189–295	2.1–3.0	4–112	1	160–503	naive	POS
	II.3	207	554	84	38	1.9–5.5	0.7–1.0	0.4–2.3	0–1.7	324 (1,300–4,300) †	263 (500–2,000) †	1.23	711 (773–1,990) †	11 (56–172) †	664 (160–920) †	ND	ND *

* All values within normal range were obtained while receiving G-CSF;

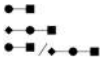
§ flow data obtained during acute meningoencephalitis were excluded;

** received rituximab historically, prior to complex immunophenotyping; rheumatoid factor (RF) is reported as positive if ever detected in peripheral blood;

† normal pediatric values for 1 year old 19, 20

Table III

MALDI-TOF quantification of glycan profiles in patients with hypomorphic *PGM3* mutations.

Patient	*O-linked*		
Normal		T antigen Sialyl-T antigen Ratio	0.22–1.14 mmol/L 11.7–31.4 mmol/L 0–0.06
I.2		T antigen Sialyl-T antigen Ratio	3.27 mmol/L 10.9 mmol/L 0.25
I.3		T antigen Sialyl-T antigen Ratio	3.82 mmol/L 28.2 mmol/L 0.12
II.1		T antigen Sialyl-T antigen Ratio	2.24 mmol/L 14.5 mmol/L 0.13
II.2		T antigen Sialyl-T antigen Ratio	5.38 mmol/L 22.8 mmol/L 0.2
II.3		T antigen Sialyl-T antigen Ratio	1.54 mmol/L 22.5 mmol/L 0.06

MALDI-TOF, Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy; PGM3, phosphoglucomutase 3; T antigen, Hex1HexNAcitol; Sialyl-T antigen Neu5AcHex1HexNAcitol.

Table IV

Comparison of conditions with elevated serum IgE versus congenital disorders of glycosylation.

	PGM3	***DOCK8***	***STAT3***	**Severe atopic dermatitis**	**CDGs**
IgE	+++	++	+++	+++	NR
Immunoglobulins	high	Low (IgM)/normal/high	low/normal	normal	low/normal
T Lymphopenia	++	++	+/–	-	+/–
Atopy	+++	+/+++	++	+++	-
Neurologic Deficits	+++	+/-	-	–	+++
EBV viremia	++	++	+	-	-
Cutaneous viral infections	++	+++	+	+	-
Lymphoma	++	++	+	-	-
Vascular Abnormality	+/-	+	++	-	-
Bronchiectasis	++	++	+++	-	-
Scoliosis	++	-	+++	+	++
Retained Primary Teeth	-	-	+++	+/–	-
Renal	++	-	+	-	+++

PGM3, phosphoglucomutase 3; DOCK8, dedicator of cytokinesis 8; STAT3, signal transducer and activator of transcription 3; atopic dermatitis, atopic dermatitis; CDGs, congenital disorders of glycosylation; NR, not reported; +, mild; ++, moderate; +++, severe; -, absent; +/–, reported but uncommon.