Autosomal recessive phosphoglucomutase 3 (*PGM3*) mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive impairment

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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: We sought to define a genetic syndrome of severe atopy, increased serum IgE levels, immune deficiency, autoimmunity, and motor and neurocognitive impairment.
Methods: Eight patients from 2 families with similar syndromic features were studied. Thorough clinical evaluations, including brain magnetic resonance imaging 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, quantitative RT-PCR, enzymatic assays, nucleotide sugar, and sugar phosphate analyses, along with matrix-assisted laser desorption/ionization–time-of-flight 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-cell numbers. Apparent brain hypomyelination resulted in markedly delayed evoked potentials and likely contributed to neurologic 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 uridine diphosphate–N-acetyl-D-glucosamine, 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. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Atopy, immune deficiency, hyper-IgE, neurocognitive impairment, phosphoglucomutase 3, glycosylation, allergy, autoimmunity*

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Mendelian atopic diseases provide opportunities to discover new pathophysiologic mechanisms contributing to allergy. Often, such diseases are identified because of features other than atopy, such as immunodeficiency (hyper-IgE syndrome, dedicator of cytokinesis 8 [DOCK8] deficiency, Omenn syndrome, Wiskott-Aldrich syndrome, and adenosine deaminase–deficient severe combined immunodeficiency), autoimmunity (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), and nonimmune abnormalities (developmental delay and abnormal skin in patients with prolidase deficiency). When they cosegregate 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 or lesions.

The prototype of such monogenic disorders is the autosomal dominant hyper-IgE syndrome, which was initially described because of recurrent staphylococcal infections[1] but was later found to be associated with increased IgE levels.[2] The identification of autosomal dominant signal transducer and activator of transcription 3 (*STAT3*) mutations in patients with hyper-IgE syndrome established a role for this transcription factor in marked IgE level increase[3,4] and, more recently, in protection from mast cell degranulation.[5] By contrast, autosomal recessive *DOCK8*

Abbreviations used
APC: Allophycocyanin
CDG: Congenital disorder of glycosylation
CFSE: Carboxyfluorescein succinimidyl ester
DOCK8: Dedicator of cytokinesis 8
GATK: Genome Analysis Toolkit
GC/MS: Gas chromatography/mass spectrometry
GlcNAc: N-acetyl-D-glucosamine
GlcNAc-1-P: GlcNAc-1-phosphate
GlcNAc-6-P: GlcNAc-6-phosphate
indel: Insertion/deletion
MRI: Magnetic resonance imaging
NIH: National Institutes of Health
PE: Phycoerythrin
PGM3: Phosphoglucomutase 3
SNV: Single nucleotide variant
STAT3: Signal transducer and activator of transcription 3
UDP: Uridine diphosphate

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 cell numbers (IL-4 and 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 level increase, the majority of patients with increased serum IgE levels 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 nonimmune abnormalities.[10]

Diseases that affect multiple organ systems, such as the one in the kindred mentioned above, include congenital disorders of glycosylation (CDGs). Typical features of CDGs 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 patients with ALG12-CDG (also called CDG-Ig) because of mutations in *ALG12*,[11] leukocyte adhesion deficiency type 2 or SLC35C1-CDG caused by 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 by using basic sugar building blocks, such as uridine diphosphate (UDP)–N-acetylglucosamine (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-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 8 patients from 2 families. The patients have severe atopy with marked serum IgE level increases, 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 might be important in the pathophysiology of allergic diseases in the general population.

METHODS

Subjects

Patients and their families provided informed consent on National Institutes of Health (NIH) institutional review board–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 NIH. Clinical immunologic laboratory tests were performed by the Department of Laboratory Medicine at the NIH (Bethesda, Md). Glycan profile quantitation and analysis in blood and urine were performed by Emory Genetics Laboratory (Decatur, Ga) using matrix-assisted laser desorption/ionization–time-of-flight mass spectroscopy.

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 magnetic resonance imaging (MRI) are provided in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Clinical phenotype of patients with hypomorphic phosphoglucomutase 3 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 3 male children of 2 consanguineous couples from Egypt was more recently identified with many phenotypic similarities (Fig 1). Both families were initially referred for evaluation because of atopic dermatitis, recurrent skin and pulmonary infections, and high serum IgE levels; *DOCK8* and *STAT3* sequences were found to be wild-type sequences.

All affected subjects had atopic dermatitis that was frequently severe, as well as atopic diatheses, including asthma; food, drug, and environmental allergies; and increased serum IgE levels. Two subjects from family I (patients I.1 and I.3) required repeated inpatient wet-wrap therapy with topical corticosteroids and emollients; both subjects displayed a marked response to therapy consistent with that seen in other patients with severe atopic dermatitis (Fig 2, *A*). Those in family I also had 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 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 lymphomas in identical twin patients I.3 and I.4, which were successfully treated with adriamycin/bleomycin/vinblastine/dacarbazine chemotherapy (Table I).