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Letter to the Editor

Defective glycosylation leads to defective gp130-dependent STAT3 signaling in PGM3-deficient patients

To the Editor:

Congenital disorders of glycosylation are a rare group of genetic disorders due to defects in protein glycosylation.

Glycosylation is a posttranslational modification essential for protein folding, cell-to-cell interactions, and proteomic diversity. Phosphoglucosyltransferase 3 (PGM3) is an enzyme necessary for the synthesis of uridine diphosphate N-acetylglucosamine, an important precursor for protein glycosylation.¹ Patients with PGM3 deficiency have a multisystemic disorder characterized by neurologic impairment and clinical features classically

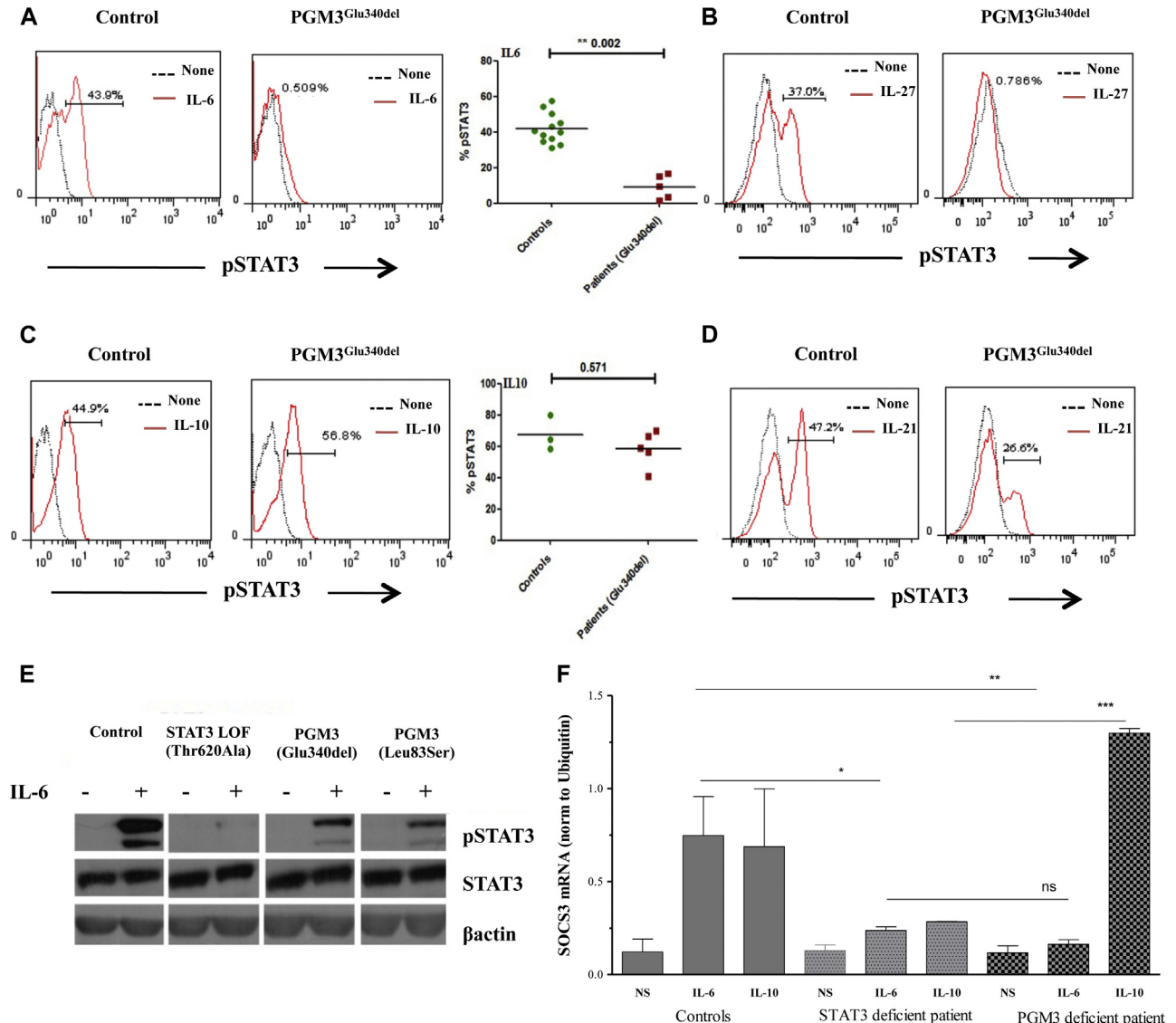


FIG 1. Impaired STAT3 phosphorylation in PGM3-deficient patients. **A**, Flow cytometric analyses of pSTAT3 (Tyr705) after stimulation with IL-6 (20 ng/mL) for 20 minutes. Results show percent phospho-STAT3⁺ cells based on the unstimulated condition. Statistical analyses were done using the Student *t* test. **P* < .05, ***P* < .01, ****P* < .001. **B**, Flow cytometric analyses of pSTAT3 (Tyr705) after stimulation with IL-27 (100 ng/mL) for 15 minutes. **C**, Flow cytometric analyses of pSTAT3 (Tyr705) after stimulation with IL-10 (20 ng/mL) for 20 minutes. **D**, Flow cytometric analyses of pSTAT3 (Tyr705) after stimulation with IL-21 (100 ng/mL) for 15 minutes. **E**, Western blot analyses of STAT3 activation (P, phosphorylation at Tyr 705) in total cell extract from EBV-transformed cell lines, produced 20 minutes after stimulation with IL-6 (20 ng/mL). Analyses were performed on 1 healthy donor, PGM3-deficient patients (p.Glu340del and p.Leu83Ser), and a STAT3-mutated patient (Thr620Ala in SH2 domain). After stripping, the blot membrane was probed with anti-STAT3 for normalization. **F**, Induction of SOCS3 mRNA in EBV-transformed cells from 4 healthy donors, 1 STAT3 LOF patient, and 1 PGM3-deficient patient (Glu340del) after 2 hours of stimulation with IL-6 (100 ng/mL) or with IL-10 (100 ng/mL). Data are displayed as $2^{-\Delta\text{CT}}$ after normalization relative to Ubiquitin (endogenous control) expression (ΔCT). LOF, Loss of function.

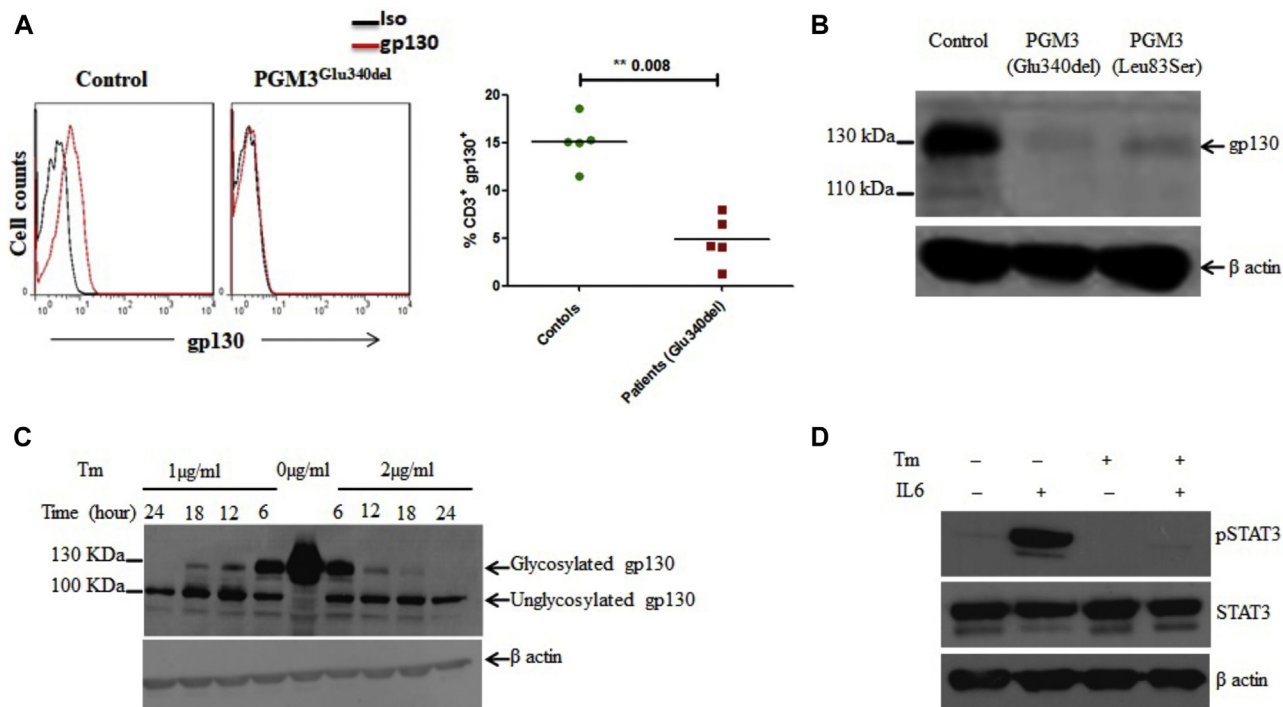


FIG 2. Defective glycosylation leads to defective gp130 expression in PGM3-deficient patients. **A**, Flow cytometry for gp130 cell surface expression in PBMCs was performed using anti-CD3 (BD Biosciences Pharmingen, San Jose, Calif) and anti-gp130 PE (BD Biosciences Pharmingen) or isotype IgG₁ (BD Biosciences Pharmingen). Statistical analyses were done using the Student *t* test. **P* < .05, ***P* < .01, ****P* < .001. **B**, Expression of gp130 was evaluated by western blot in denatured whole-cell lysates from EBV-transformed cell lines, using anti-gp130 antibody (Cell Signaling Technology, Danvers, Mass). Analyses were performed on 1 healthy donor and PGM3-deficient patients (p.Glu340del and p.Leu83Ser). **C**, Kinetic of gp130 deglycosylation using Tunicamycin (Tm) with the indicated concentrations. After each treatment, cell lysates were prepared and immunoblotted with anti-gp130 (cell signaling). **D**, Tunicamycin (Tm) inhibits the activation of STAT3 by IL-6. EBV-transformed cell lines of healthy control were pretreated with 1 μg/mL of tunicamycin for 6 hours and stimulated with IL-6 (20 ng/mL) for 20 minutes.

associated with autosomal-dominant hyper-IgE syndrome (AD-HIES) including recurrent pneumonias, skin abscesses, elevated levels of IgE, and abnormalities in connective tissues and bones.²⁻⁴ AD-HIES is caused by dominant negative mutations in the gene encoding signal transducer and activator of transcription 3 (STAT3), used for signaling by a wide range of cytokines and growth factors. We demonstrate that patients with PGM3 deficiency have impaired STAT3 signaling downstream of the highly glycosylated protein gp130, the shared signal transducing receptor subunit for IL-6-type cytokines, thereby constituting the mechanistic basis for the clinical features shared by PGM3 deficiency and AD-HIES.

We studied 6 patients from 3 kindreds with PGM3 deficiency. The previously described mutations, p.Glu340del and p.Leu83Ser, were found in 5 patients and 1 patient, respectively.^{4,5} The patients had recurrent pneumonias, skin abscesses, increased serum IgE levels, candidiasis, and skeletal abnormalities. In addition, they had moderate-to-severe neurologic impairment. IL-6-driven STAT3 phosphorylation was impaired in PBMCs and EBV-transformed B cells from the patients (Fig 1, A and E). Cellular response to IL-27, another gp130-dependent cytokine, was impaired as well (Fig 1, B). In contrast, patients had normal STAT3 phosphorylation in response to IL-10 and IL-21, 2 independent gp130 cytokines (Fig 1, C and D; see Fig E1 in this article's Online Repository at www.jacionline.org). Our results are consistent with those of a recent study of gp-

130-deficient patient who presented with clinical features typical of AD-HIES and showed STAT3 phosphorylation impairment in response to different members of the IL-6 family of cytokines.⁶ The induction of SOCS3 target gene in patient's EBV-B-cell lines was impaired in response to IL-6 stimulation (*P* value = .0053), whereas it was normal in response to IL-10 stimulation (Fig 1, F), confirming that IL-6-driven gp130-mediated STAT3 phosphorylation is impaired in PGM3-deficient patients. In addition, despite normal IL-17-producing T cells in PGM3-deficient patients when studied *ex vivo*,⁵ we tested whether there is an impairment of *in vitro* differentiation of naive CD45RA⁺/CD4⁺ T cells into IL-17-producing T cells. We were unable to detect IL-17A-expressing T cells by intracellular staining in a PGM3^{Glu340del} patient when naive CD4 T cells were cultured under T_H17-polarizing conditions (see Fig E2 in this article's Online Repository at www.jacionline.org).

Gp130 has 9 N-glycosylated sites. N-linked glycosylation is crucial for its stability; unglycosylated gp130 undergoes proteasomal degradation before reaching the cell surface.⁷ We hypothesized that gp130 would be weakly expressed on PGM3-deficient cells, due to impaired glycosylation. Flow cytometry and immunoblotting showed significantly lower gp130 expression in peripheral T cells and EBV-transformed B cells from PGM3-deficient patients compared with healthy donors (Fig 2, A and B). Patients with PGM3^{Glu340del}, known to cause a severe phenotype, had less expression of gp130 than did

those with PGM3^{Leu83Ser} (Fig 2, A and B), a finding attributed to the more profound impairment of glycosylation caused by the p.Glu340del mutation.⁴ PGM3 gene silencing will definitely further confirm the role of PGM3-dependent glycosylation in gp130 signaling. We also assessed the effect of tunicamycin, an inhibitor of N-glycosylation, on gp130-mediated signaling in EBV cell lines from a healthy donor. Tunicamycin treatment gradually replaced the glycosylated form of gp130 (~130 kDa) with its unglycosylated form (100 kDa) and inhibited STAT3 activation in response to IL-6 (Fig 2, C and D). The absence of the lower 100-kD band, corresponding to the unglycosylated gp130 isoform in the patients' lysates (Fig 2, B), is likely due to proteasomal degradation, as has been seen in another study.⁷ Our results are consistent with the observation that N-glycosylation inhibition of gp130 abolishes IL-6-driven STAT3 activation in cultured cardiac myocytes.⁸

Collectively, our findings demonstrate that defective glycosylation in PGM3-deficient patients results in reduced expression of unglycosylated gp130 protein and consequently, impaired gp130-dependent STAT3 phosphorylation. This may account for the overlapping clinical features shared by PGM3 deficiency, AD-HIES, and gp130 deficiency. Indeed, we have shown that PGM3-deficient patients have defective IL-6 and IL-27 signaling. Impaired IL-6/gp130/STAT3 signaling in PGM3-deficient patients could explain in part low IL-17-producing T cells generated *in vitro* as well as low memory B cells. In addition, the impairment of T-cell proliferation and the increased proportion of T_H2 cells observed in PGM3-deficient patients could be attributed to aberrant IL-27 signaling. Indeed, several reports showed that IL-27 plays a critical role in the suppression of T_H2 responses.⁹ Altogether, these functional cellular phenotypes observed in PGM3-deficient patients overlap with STAT3 loss of function and gp130-deficient patients and mechanistically tie the PGM3 deficiency to poor gp130-dependent STAT3 signaling.

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REFERENCES

- Greig KT, Antonchuk J, Metcalf D, Morgan PO, Krebs DL, Zhang J-G, et al. Agm1/Pgm3-mediated sugar nucleotide synthesis is essential for hematopoiesis and development. *Mol Cell Biol* 2007;27:5849-59.
- Zhang Y, Yu X, Ichikawa M, Lyons JJ, Datta S, Lamborn IT, et al. Autosomal recessive phosphoglucomutase 3 (PGM3) mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive impairment. *J Allergy Clin Immunol* 2014;133:1400-9, 1409.e1-5.
- Stray-Pedersen A, Backe PH, Sorte HS, Mørkrid L, Chokshi NY, Erichsen HC, et al. PGM3 mutations cause a congenital disorder of glycosylation with severe immunodeficiency and skeletal dysplasia. *Am J Hum Genet* 2014;95:96-107.
- Sassi A, Lazaroski S, Wu G, Haslam SM, Fliegau M, Mellouli F, et al. Hypomorphic homozygous mutations in phosphoglucomutase 3 (PGM3) impair immunity and increase serum IgE levels. *J Allergy Clin Immunol* 2014;133:1410-9, 1419.e1-13.
- Ben-Khemis L, Mekki N, Ben-Mustapha I, Rouault K, Mellouli F, Khemiri M, et al. A founder mutation underlies a severe form of phosphoglucomutase 3 (PGM3) deficiency in Tunisian patients. *Mol Immunol* 2017;90:57-63.
- Schwerd T, Twigg SRF, Aschenbrenner D, Manrique S, Miller KA, Taylor IB, et al. A biallelic mutation in IL6ST encoding the GP130 co-receptor causes immunodeficiency and craniosynostosis. *J Exp Med* 2017;214:2547-62.
- Waetzig GH, Chalaris A, Rosenstiel P, Suthaus J, Holland C, Karl N, et al. N-linked glycosylation is essential for the stability but not the signaling function of the interleukin-6 signal transducer glycoprotein 130. *J Biol Chem* 2010;285:1781-9.
- Matsuo R, Morihara H, Mohri T, Murasawa S, Takewaki K, Nakayama H, et al. The inhibition of N-glycosylation of glycoprotein 130 molecule abolishes STAT3 activation by IL-6 family cytokines in cultured cardiac myocytes. *PLoS One* 2014;9:e111097.
- Yoshida H, Hunter CA. The immunobiology of interleukin-27. *Annu Rev Immunol* 2015;33:417-43.

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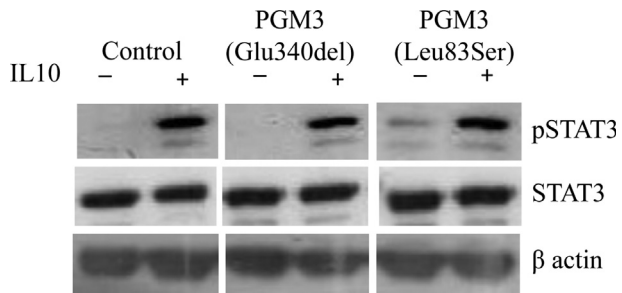


FIG E1. Normal STAT3 phosphorylation in response to IL10 in EBV-transformed cell lines from p.Glu340del and p.Leu83Ser PGM3-deficient patients.

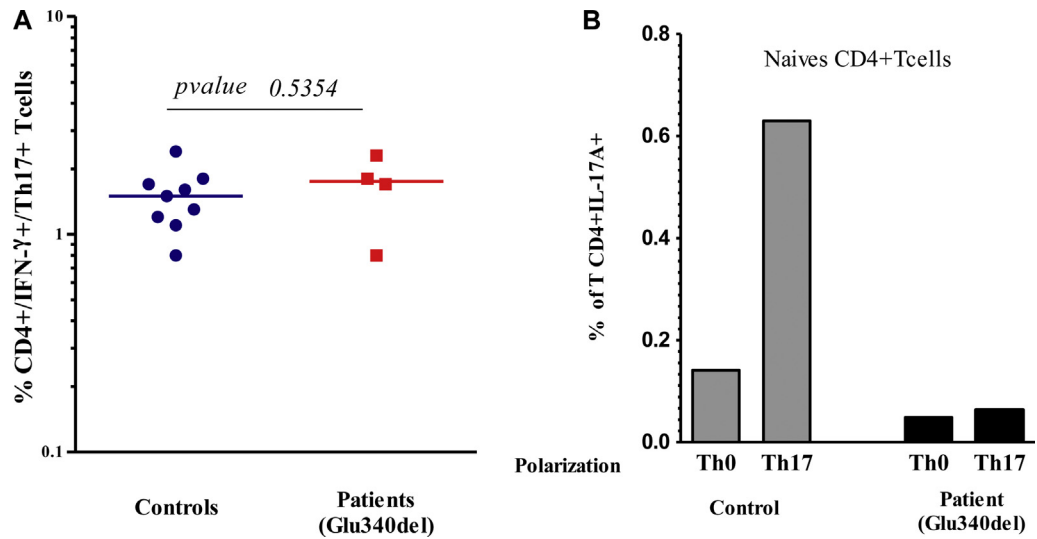


FIG E2. Impaired T_H17 differentiation in PGM3-deficient patient. **A**, Frequency of T_H17 subset among $CD3^+IL-17A^+$ cells evaluated *ex vivo* in patients and controls. Horizontal bars indicate medians. **B**, Frequency of T_H17 subset after *in vitro* differentiation of naive $CD4^+$ T cells. Naive $CD45RA^+CD4^+$ T cells were isolated from nonadherent PBMCs by immune-magnetic depletion of $CD4^-CD45RO^+$ T cells (purity > 95%) and cultured under Th0 conditions (CD3-CD28) or T_H17 -polarizing conditions (IL-1 β , IL-23, and IL-6) for 4 days.