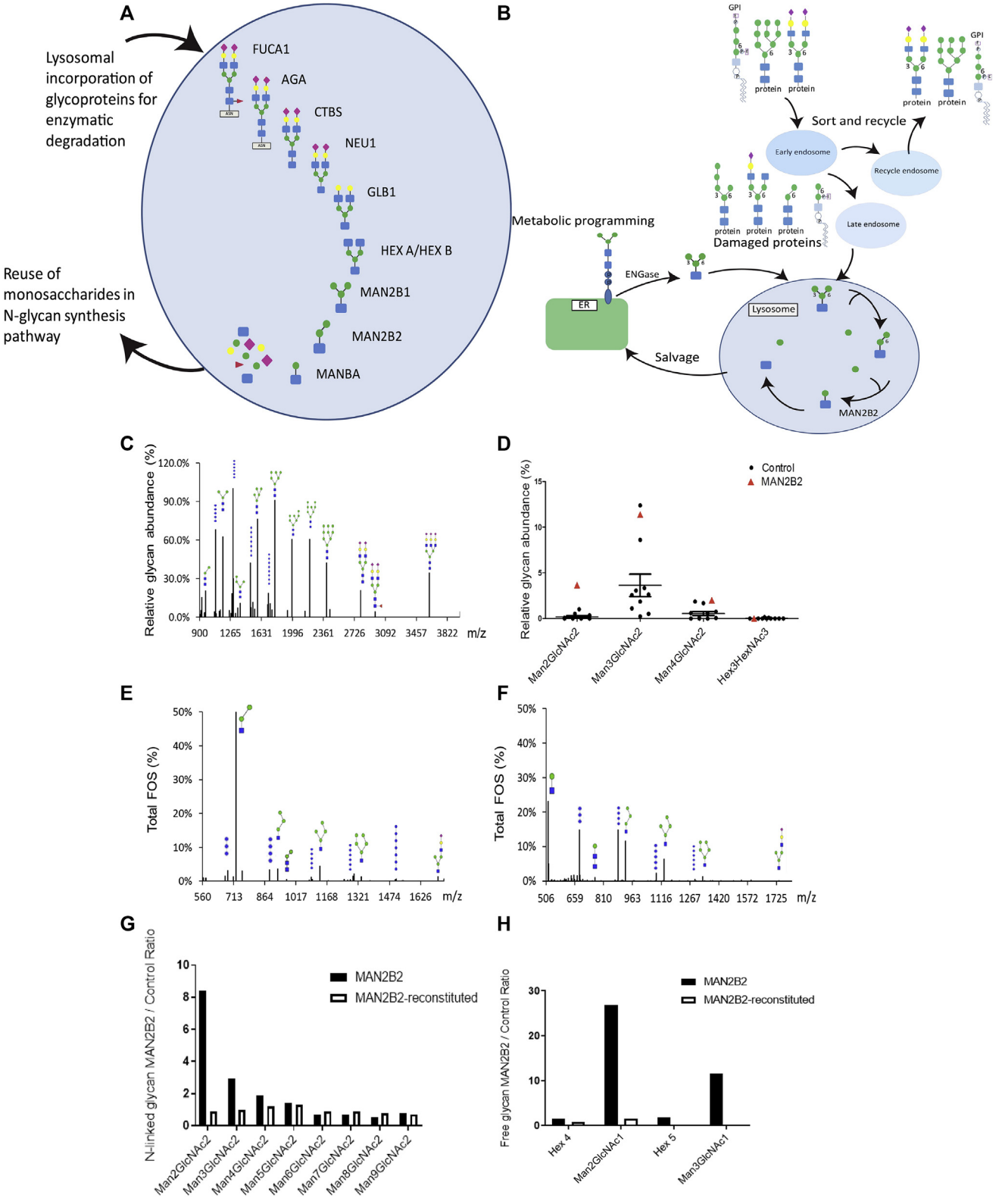
Defining a new immune deficiency syndrome: MAN2B2-CDG

To the Editor:

Congenital disorders of glycosylation (CDGs) are a group of clinically heterogeneous disorders characterized by abnormal monosaccharide activation and protein and lipid glycosylation.[1] More than 147 CDG subtypes have currently been described to affect several glycosylation pathways, including *N*-glycosylation, *O*-glycosylation, glycosaminoglycan, dystroglycanopathy, and glycosylphosphatidylinositol (GPI)-anchor pathways.[1]

Several CDG subtypes involve enzymatic deficiencies in the *N*-linked glycosylation pathway. *N*-linked protein glycosylation is a highly conserved process occurring in the endoplasmic



reticulum (ER) and Golgi compartments. In this pathway, oligosaccharyltransferase initiates the synthesis process by transfer of a lipid-linked oligosaccharide sugar to asparagine residues flanked by consensus amino acid sequence N-X-S/T in the target protein.[1] Subsequent steps involve addition and trimming of glycans from the sugar chain, including mannose, galactose, fucose, and glucose. Deficiency of ER and Golgi enzymes catalyzing the glycosylation steps gives rise to various CDGs with highly variable phenotypes. In addition, many CDGs show disruption in lysosomal pathways.[2]

Here, we present the functional and metabolic studies on a patient with combined immune deficiency harboring biallelic mutations in the mannosidase alpha class 2B member 2 (*MAN2B2*) gene, affecting both *N*-glycan synthesis and glycan degradation (for detailed description of the clinical phenotype and of methods, see this article's Online Repository at www.jacionline.org). The patient is an 8-year-old girl of consanguineous healthy parents from Saudi Arabia. She was born at term. Starting at age 2 weeks, she suffered from recurrent pneumonias and thrush. She manifested small-vessel vasculitis affecting fingers, toes, and ears starting about age 3 months and arthritis of the knees, hips, and wrists starting at age 9 months. At age 5 months, the thymus shadow was not visualized at chest X-ray. At that time, she had normal IgG (7.8 g/L), IgA (1.28 g/L), and IgM (0.88 g/L) levels, but elevated serum IgE (42,550 kU/L). The patient had a thrombotic stroke and left hemiparesis around age 16 months. She also developed chronic diarrhea requiring total parenteral nutrition and nasogastric tube feeds, recurrent oral herpes, and severe failure to thrive. Psychomotor developmental delay was noted. She continued to have recurrent respiratory infections that required intubation at age 16 and 24 months, and initiation of immunoglobulin replacement therapy. Physical examination at the age of 4 years revealed microcephaly (45.5 cm, −2.5 SDs), low height (84.5 cm; *Z* score, −5.6) and weight (9.4 kg; *Z* score, −4.52), strabismus, beaked nose, hyperextensible skin on the back of the hands, pectus carinatum, visible veins on the abdominal skin, and mild hepatomegaly. A brain magnetic resonance imaging showed a large chronic infarct of the right frontoparietal region with encephalomalacia, gliosis, and areas of laminar necrosis and hemosiderin deposition– associated right midbrain Wallerian degeneration (see Fig E1 in this article's Online Repository at www.jacionline.org). She had significant speech delay and was able to walk only with support. She continued to have recurrent flares of vasculitis and arthritis, with elevated C-reactive protein and erythrocyte sedimentation rate, and positivity for von Willebrand factor antigen and for rheumatoid factor. Laboratory studies at age 5 years revealed anemia (hemoglobin, 8.7 g/dL), thrombocytopenia (platelet count, 78×10^9/L), and lymphopenia (520 cells/μL), with low T- and B-cell counts, low proportion of naive $CD4^+$ and $CD8^+$ cells, markedly increased proportion (92.9%) of terminally differentiated, $CD45RA^+$ $CCR7^-$ (T_{EMRA}) $CD8^+$ cells, skewed repertoire of $CD8^+$ T cells, undetectable levels of T-cell receptor excision circles, and impaired *in vitro* T-cell proliferation to mitogens and antigens (see Table E1 and Fig E2 in this article's Online Repository at www.jacionline.org). Maternal T-cell engraftment was ruled out. The proportion of switched memory B cells was normal (11.2%), but there was an elevated percentage of circulating plasmablasts (7.4%) and of dysreactive $CD21^{low}$ $CD38^{low}$ B cells (53.8%). Clinical and laboratory findings were suggestive of a CDG. Whole-exome sequencing (WES) identified a homozygous missense variant in the *MAN2B2* gene (Chr4:g.6575322G>A; p.Asp38Asn), which segregates with disease in the pedigree. Both parents are healthy unaffected heterozygous carriers for p.Asp38Asn. Two of 3 patient siblings are healthy unaffected heterozygous carriers for p.Asp38Asn, 1 healthy unaffected sibling is homozygous for the wild-type *MAN2B2* allele (see Fig E3, *A* and *B*, in this article's Online Repository at www.jacionline.org). The MAN2B2 Asp38 residue is conserved among several mammalian species and zebrafish (Fig E3, *C*). In-silico tools all predict pathogenic/deleterious effects of p.Asp38Asn. (Fig E3, *D*). Querying of the Genome Aggregation Database indicates heterozygous carrier status for p.Asp38Asn in 62 individuals (allele count 62 in 230,700 total alleles; minor allele frequency [MAF], 0.0002687). Importantly, no p.Asp38Asn homozygotes have been reported. The combined annotation-dependent depletion score for this variant is 28.300, significantly higher than the Mutation Significance Cutoff score, which for the *MAN2B2* gene is 3.313. In-silico protein modeling indicates a change in Gibbs free energy ($\Delta\Delta G$), resulting in altered protein stability for p.Asp38Asn. ΔΔG for the mutant protein was observed higher in lysosomal pH range (pH 4; $\Delta\Delta G$, 2.13) compared with cytosolic pH range (pH 7; $\Delta\Delta G$, 1.13) (Fig E3, *D*). A list of the other rare (MAF < 0.001) homozygous variants identified by WES is provided in Table E2 in this article's Online Repository at www.jacionline.org; none of them have been associated with immunodeficiency or immune dysregulation. To further assess the possible role played by these variants, WES was also performed in both parents and in 2 of the unaffected siblings (III,2 and III,3 in Fig E3, *A*), and the rare variants shared by the proband and by these unaffected family members are reported in Table E2.

FIG 1. Abundance of Man2GlcNAc2 and Man2GlcNAc1 in patient fibroblasts is reversed upon lentivirus-mediated transfer of wild-type MAN2B2. **A,** Schematic representation of the lysosomal *N*-glycan degradation and monosaccharide salvage pathway. Released monosaccharides are reused as source for glycosylation in ER and Golgi. Figure adapted from Suzuki.[3] **B,** Sorting and recycling pathways of *N*-linked and GPI-anchored glycoproteins, and subsequent salvage pathway feeding into glycan biosynthesis. **C,** MALDI TOF spectra of permethylated *N*-glycans from patient fibroblasts, showing abundant glycans. **D,** Quantification of relative abundance of glycans in patient and control fibroblasts. Values are presented from MALDI TOF permethylated *N*-glycan profiles for patient fibroblasts and 10 controls. Glycan identity is indicated for Man2GlcNAc2. Blue square: *N*-acetylglucosamine (GlcNAc); green circle: mannose. **E,** MALDI TOF free glycan profiling shows increased abundance of Man2GlcNAc1 in patient fibroblasts. FOS: free oligosaccharide. **F,** Enzymatic 1,6 mannosidase digestion shows trimming of Man2GlcNAc1 to Man1GlcNAc1, indicating that the pool of Man2GlcNAc1 accumulated in patient fibroblasts carries core 1,6 mannose residues. **G,** *N*-linked glycan profiling in patient fibroblasts transduced with wild-type MAN2B2 lentiviral vector shows reduction of Man2GlcNAc2 and Man3GlcNAc2. **H,** Free glycan profiles in patient fibroblasts transduced with wild-type MAN2B lentiviral vector show reduction in relative abundance of Man2GlcNAc1. *MALDI-TOF,* Matrix-assisted laser desorption/ionization time-of-flight.

MAN2B2 is a member of the mannosidase gene family involved in the lysosomal degradation of glycoproteins. Lysosomal processing of glycoproteins is central to catabolism of glycoproteins and an important regulatory mechanism for homeostasis of glycosylation.[3] Mature glycoproteins enter the lysosomal degradation pathway, where a range of enzymes catabolize the conversion of glycoproteins to amino acids and monosaccharides. Free monosaccharides derived from lysosomal degradation enter the recycling or salvage pathway of monosaccharides and constitute an important source for glycans in the ER and Golgi.[4]

Degradation of glycoproteins in the lysosome is mediated through several enzymes involved in reduction of distinct monosaccharides (Fig 1, *A*). Demannosylation of free *N*-glycans and completion of the lysosomal glycoprotein degradation pathway is mediated by enzymatic activity of alpha- and beta-mannosidase enzymes. Within the mannosidase gene family, lysosomal alpha-mannosidase (MAN2B1), core specific lysosomal alpha-1,6 mannosidase (MAN2B2), and mannosidase beta (MANBA) are recruited for the final steps of lysosomal glycoprotein degradation.

Loss of MAN2B1 and of MANBA enzymatic activity results in alpha-mannosidosis (MIM# 248500)[5] and beta-mannosidosis (MIM# 248510),[6] respectively. In contrast, loss of MAN2B2 has currently not been reported causative for any disorder. *MAN2B2* represents a relatively understudied gene, for which expression profiles and enzymatic role have been elucidated only recently.[7] MAN2B2 is a lysosomal alpha-mannosidase specific for cleavage of the α1-6-mannose residue of *N*-linked glycans, and cleaves the Chitobiase (CTBS) product Man2GlcNAc1 to generate Man1GlcNAc1 (Fig 1, *B*).

Real-time quantitative polymerase chain reaction analysis did not show altered *MAN2B2* mRNA expression in patient versus control fibroblasts (data not shown). Because the p.Asp38Asn missense variant is localized within the zinc-binding region of MAN2B2 (Fig E3, *E*), loss of zinc-binding affinity could putatively disrupt enzymatic activity and cause pathogenicity, as observed for the MAN2B1 variant p.D74E associated with alpha-mannosidosis.[8]

Serum *N*-glycan profiling by electrospray-ionization quadrupole time-of-flight indicated elevated Man5/Man6 and Man5/Man9 in the patient (see Table E3 in this article's Online Repository at www.jacionline.org). The effect of MAN2B2 p.Asp38Asn variant on *N*-glycosylation and glycan degradation was investigated by *N*-linked and free glycan profiling in patient and control fibroblasts by using mass spectrometry. In patient fibroblasts, *N*-linked glycan profiling showed marked accumulation of Man2GlcNAc2 glycans compared with profiles obtained in fibroblast cells from 10 healthy controls (Fig 1, *C* and *D*). Free glycan profiling indicated high abundance of Man2GlcNAc1, in addition to high abundance of Man3GlcNAc1, consistent with glycosylation profiles observed in cells defective in glycoprotein degradation (Fig 1, *E*). Enzymatic digestion of 1,6 mannose in patient fibroblasts through α-1,6-mannosidase treatment showed digestion of accumulated Man2GlcNAc1 to Man1GlcNAc1 (Fig 1, *F*), indicating that the accumulated Man2GlcNAc1 in patient cells carries a terminal 1,6-mannose motif. Accumulation of Man2GlcNAc1 indicates abnormal lysosomal function in MAN2B2-deficient cells.

Lentiviral transduction of wild-type *MAN2B2* in patient fibroblasts led to normalization of the *N*-linked glycan profile (Fig 1, *G*; see Table E4 in this article's Online Repository at www.jacionline.org). The relative abundance of Man2GlcNAc2 was reduced from 8.4 times control levels in patient fibroblasts to 0.9 times control levels in wild-type *MAN2B2*-transduced patient fibroblasts. Man3GlcNAc2 was reduced from 2.9 control levels in patient fibroblast to 1.0 times control levels in wild-type *MAN2B2*-transduced patient fibroblasts. In addition, free glycan profiling showed reduction of Man2GlcNAc1 in wild-type MAN2B2-transduced fibroblasts, with a decrease in relative abundance of Man2GlcNAc1 from 26.9 to 1.5 times control levels, indicating rescue of the impaired deglycosylation of Man2GlcNAc1 in wild-type *MAN2B2*-transduced patient fibroblast cells (Fig 1, *H*; Table E4).

Western blotting of glycosylation levels of intercellular adhesion molecule 1 was applied to analyze cellular *N*-glycosylation. A strong reduction in intercellular adhesion molecule 1 (ICAM-1) glycosylation was observed in patient fibroblasts compared with control (see Fig E4, *A*, in this article's Online Repository at www.jacionline.org). Furthermore, hypoglycosylation of lysosome-associated membrane protein 2 (LAMP2) in patient fibroblasts was indicative of altered *N*-glycosylation (Fig E4, *B*). Lentiviral transduction of wild-type MAN2B2 into patient fibroblasts rescued glycosylated ICAM-1 and LAMP2 levels (Fig E4, *C* and *D*).

Overall, our results indicate that loss of MAN2B2 enzymatic activity and subsequent impairment of α1,6-mannosidosis leads to dysregulation of deglycosylation and abnormal mannosylation of glycans. The clinical presentation of the MAN2B2-deficient patient, with immune deficiency, immune dysregulation, failure to thrive, strabismus, and neurodevelopmental disability is highly overlapping with features frequently seen in CDG. At least 23 CDGs, affecting either early (ER) or late (Golgi)-related disruptions of glycosylation, include immune deficiency as a prominent phenotype.[9] Although our data indicate that MAN2B2 deficiency should be added to the list of CDGs, additional investigations are warranted to further elucidate the full spectrum of MAN2B2 activity and localization. Because 1,6-mannose is also a core component of GPI anchors, whether the sorting and degradation of GPI-anchored proteins is impaired in MAN2B2 deficiency may further our understanding of the pathogenesis. Putative impairment of the *N*-glycan synthesis pathway could be analyzed in more detail through delay of glycoproteins trafficking between ER and Golgi. By performing WES also in several unaffected family members, we have restricted the number of homozygous genetic variants that are unique to the patient. It is possible that some of them may have contributed to the disease phenotype, in particular the variants in the *CIT* gene (associated with microcephaly) and in *TRAPPC9* (involved in regulation of nuclear factor kappa B signaling). Based on the functional studies performed, *MAN2B2* is the only gene responsible for the metabolic phenotype. Furthermore, immune deficiency has been reported in patients with various forms of CDG, whereas none of the other rare homozygous variants identified in the patient have been previously described in patients with immune defects. These data strongly suggest that the disease phenotype is largely due to the *MAN2B2* variant, although a contributory role of other variants cannot be excluded. Identification of additional patients harboring pathogenic variants in *MAN2B2* is crucial to gain further insight into the clinical phenotype spectrum of MAN2B2 deficiency and to validate the association between MAN2B2 deficiency and immune dysfunction.

In conclusion, we have reported a syndromic patient with combined immune deficiency and a homozygous *MAN2B2* variant p.Asp38Asn associated with abnormalities of glycosylation and lysosomal involvement that were reversed *in vitro* upon lentivirus-mediated transfer of wild-type MAN2B2. We propose *MAN2B2* biallelic p.Asp38Asn as a novel pathogenic variant leading to combined immune deficiency, abnormal glycosylation, and lysosomal involvement. Patients have normal transferrin isoelectric focusing profiles, and mild glycosylation changes by electrospray-ionization quadrupole time-of-flight in blood. At the age of 5 years, the patient received hematopoietic stem cell transplantation from her phenotypically HLA-matched father after reduced-intensity conditioning with fludarabine, busulfan, and rabbit antithymocyte globulin. Six months after hematopoietic stem cell transplantation, improvement in T-cell count and function, and in immunoglobulin production (with independence from intravenous immunoglobulins), was observed (Table E1), and stabilization of the disease was achieved, with resolution of infections. Our findings imply MAN2B2 deficiency as a novel CDG. On the basis of our data, we recommend screening for putative pathogenic variants in the *MAN2B2* gene in pediatric patients presenting with combined immune deficiency and severe growth delay with intellectual or developmental disability.

Jan Verheijen, PhD[a]
Sunnie Y. Wong, MD, PhD[b]
Jared H. Rowe, MD, PhD[c]
Kimiyo Raymond, MD[d]
Jennifer Stoddard, PhD[e]
Ottavia M. Delmonte, MD, PhD[f]
Marita Bosticardo, PhD[f]
Kerry Dobbs, BS[f]
Julie Niemela, PhD[e]
Enrica Calzoni, MD[f]
Sung-Yun Pai, MD[g,h]
Uimook Choi, PhD[f]
Yasuhiro Yamazaki, MD, PhD[f]
Anne Marie Comeau, PhD[i]
Erin Janssen, MD, PhD[c]
Lauren Henderson, MD, MMSc[c]
Melissa Hazen, MD[c]
Gerard Berry, MD[j]
Sergio D. Rosenzweig, MD, PhD[e]
Hasan Hamdan Aldhekri, MD[k]
Miao He, PhD[l]
Luigi D. Notarangelo, MD[f]
Eva Morava, MD, PhD[a]

From [a]the Center for Individualized Medicine, Department of Clinical Genomics, Mayo Clinic, Rochester, Minn; [b]Hayward Genetics Center, Tulane University Medical School, New Orleans, La; [c]the Division of Immunology, Boston Children's Hospital, Boston, Mass; [d]the Biochemical Genetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, Minn; [e]the Immunology Service, Department of Laboratory Medicine, National Institutes of Health, Bethesda, Md; [f]the Laboratory of Clinical Immunology and Microbiology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md; [g]the Division of Hematology/Oncology, Boston Children's Hospital, Boston, Mass; [h]the Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, Mass; [i]the New England Newborn Screening Program and the Department of Pediatrics, University of Massachusetts Medical School, Worcester, Mass; [j]the Division of Genetics and Genomics, Department of Pediatrics, Boston Children's Hospital, Boston, Mass; [k]the Section of Pediatric Allergy and Immunology, King Faisal Hospital and Research Center, Riyadh, Saudi Arabia; and [l]Palmieri Metabolic Disease Laboratory, The Children's Hospital of Philadelphia, Philadelphia, Pa. E-mail: hem@email.chop.edu. Or: luigi.notarangelo2@nih.gov. Or: Morava-Kozicz.Eva@Mayo.edu.

This work was supported in part by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health (to L.D.N.) and by grant number U54 NS115198-01 (to E.M.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Available online November 24, 2019.
https://doi.org/10.1016/j.jaci.2019.11.016

Defining the biological responses of IL-6 by the study of a novel IL-6 receptor chain immunodeficiency

To the Editor:

Patients with primary immunodeficiency and atopy were found to have genetic faults in various components of the IL-6 pathway.[1,2] IL-6 has a wide range of biological actions and target tissues.[3] The cell-surface receptor for IL-6 is composed of 2 transmembrane chains: a ligand binding IL-6 receptor (IL-6R) and the signal-transducing gp130 (IL6ST) coreceptor. Binding of IL-6 to IL-6R triggers association with gp130 and formation of a high-affinity IL-6 binding site, initiating signal transduction. Janus kinases are recruited to gp130, mediating activation of signal transducer and activator of transcription (STAT) 1 and STAT3. IL-6 can alternatively signal through a soluble IL-6R–IL-6 complex binding to cell surface–expressed gp130 in a process known as trans-signaling.[4] Although the IL-6R chain is specific for IL-6, gp130 is a shared component of many cytokine receptor complexes.

The majority of known genetic defects related to the IL-6 pathway have not been specific for IL-6R-mediated responses but rather occur in the context of multiple receptor-signaling defects, as seen with gp130 loss-of-function mutations[5] or in hyper-IgE syndrome caused by *STAT3* mutations.[2] These are complex immunodeficiencies presenting with recurrent infections, increased IgE levels, eosinophilia, and skeletal and connective tissue abnormalities. Most recently, Spencer et al[6] described mutation of the IL-6R chain resulting in recurrent infection, severe eczema, and increased IgE levels.