Author Manuscript

HHS Public Access
Author manuscript
J Allergy Clin Immunol. Author manuscript; available in PMC 2024 October 29.

Published in final edited form as:
J Allergy Clin Immunol. 2020 June ; 145(6): 1696–1699.e6. doi:10.1016/j.jaci.2020.01.014.

Severe combined immunodeficiency caused by inositol-trisphosphate 3-kinase B (ITPKB) deficiency

Abduarahman Almutairi, MD[a,*], Jacqueline G. Wallace, BSPH[a,*], Faris Jaber, MD[a], Mohammed F. Alosaimi, MD[a,b], Jennifer Jones, BA[a], Mohamed T. H. Sallam, MD, PhD[c], M. H. Elnagdy, MD, PhD[d], Janet Chou, MD[a,‡], Ali Sobh, MD[e,‡], Raif S. Geha, MD[a,‡]

[a]the Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, Mass

[b]the Department of Pediatrics, Allergy and Immunology Division, King Saud University, Riyadh, Saudi Arabia

[c]the Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo

[d]the Department of Medical Biochemistry, Faculty of Medicine, Mansoura University, Mansoura

[e]the Department of Pediatrics, Mansoura University Children's Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt

To the Editor:

Inositol phosphate 3-kinases (ITPKs) regulate inositol phosphate signaling downstream of TCR activation. Antigen receptor engagement activates phosphoinositide 3-kinase, which in turn phosphorylates the membrane-bound lipid phosphatidylinositol(4,5) bisphosphate to generate phosphatidylinositol(3,4,5) trisphosphate (PIP3). PIP3 binds to the pleckstrin homology domains of ITK/TEC-, PDK1-, and AKT-family kinases, resulting in their membrane recruitment and activation. Receptor signaling also activates phospholipase C-γ, which hydrolyzes phosphatidylinositol(4,5) bisphosphate to generate inositol 1,4,5-trisphosphate (IP3).[1] The binding of IP3 to its receptor on the endoplasmic reticulum releases calcium from the endoplasmic reticulum into the cytosol, thereby inducing calcium influx through store-operated calcium entry plasma membrane channels (Fig 1, A). ITPKs convert IP3 into inositol 1,3,4,5-tetrakisphosphate (IP4).[1] IP4 enhances PIP3 binding to ITK, thus enhancing ITK activation and establishing a feedback loop of phospholipase C-g1 activation essential for T-cell development.[2,3] IP4 also regulates inositol signaling by competing with membrane-bound PIP3 for binding to AKT, thereby limiting AKT activation.[1] IP4 dampens store-operated calcium entry, thereby inhibiting calcium-dependent apoptotic pathways, and binds to the GTPase GAP1[IP4BP] to activate Ras and Rap to promote positive selection of thymocytes.

raif.geha@childrens.harvard.edu.
*Equal first authors.
‡Equal last authors.

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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

There are 3 ITPK isoforms: ITPKA is expressed solely in neurons, whereas ITPKB and ITPKC are ubiquitous. They share highly homologous kinase domains at their C-terminus,[1] but differ in their N-terminus domains, enabling distinct subcellular localization and protein interactions. *Itpkb*$^{-/-}$, but not Itpkc$^{-/-}$, mice have defective thymic development, severe peripheral T-cell lymphopenia, and decreased peripheral B cells.[4,5] We report the first patient with ITPKB deficiency associated with a T^{-}B^{+}NK^{+} severe combined immunodeficiency phenotype.

The daughter of consanguineous Egyptian parents developed failure to thrive, persistent thrush shortly after birth, recurrent pneumonias beginning at age 2 months, and *Klebsiella pneumoniae* skin abscesses at age 6 and 10 months. There was no evidence of organ abnormalities. At age 6 months, the absence of a thymic shadow was noted on chest radiograph and laboratory investigation revealed anemia, leukopenia, neutropenia, thrombocytopenia, and profound CD3^{+} CD4^{+} and CD8^{+} cell lymphopenia (Table I; see Table E1 in this article's Online Repository at www.jacionline.org). CD19^{+} and CD56^{+} natural killer (NK)-cell numbers were normal. Serum IgG, which at age 6 months may reflect the contribution of transplacental maternal IgG, was borderline low; serum IgA and IgM were normal. Serial determinations showed persistent anemia, neutropenia, thrombocytopenia, and lymphopenia (Table E1). No additional immunophenotyping studies were available on the patient. She was treated with immunoglobulin replacement and antibiotics, and died at age 11 months with severe pneumonia and *Staphylococcus aureus* sepsis. The clinical and laboratory findings are consistent with T^{-}B^{+}NK^{+} severe combined immunodeficiency. The patient's mother, father, and brother had no history of recurrent infections. CD3^{+}, CD4^{+}, CD8^{+}, and CD19^{+} B-cell numbers were normal in the parents (see Table E2 in this article's Online Repository at www.jacionline.org).

A targeted next-generation sequencing panel for 264 genes associated with primary immunodeficiency, performed at age 10 months, revealed no candidate variants. Whole-exome sequencing identified a homozygous frameshift variant in *ITPKB* (c.2583delT, p.Arg862Glufs*4). The mutation is not present in the 1000 Genomes, ExAC, or NHLBI Exome Sequencing Project databases. In addition, no loss-of-function variants in *ITPKB* have been reported in the ExAC database, indicating the significant rarity of deleterious *ITPKB* mutations in the general population. Sanger sequencing revealed that the *ITPKB* mutation is homozygous in the patient and heterozygous in the parents and brother (see Fig E1 in this article's Online Repository at www.jacionline.org). The variant is predicted to delete 84 C-terminal amino acids in the kinase domain (Fig 1, C and D). Other than *ITPKB*, no mutations in genes known or suspected to be associated with immunodeficiency were detected (see Table E3 in this article's Online Repository at www.jacionline.org).

Because of the patient's death, additional studies of the patient's cells were not possible. Therefore, to assess the impact of the patient's mutation on protein expression, HEK293T cells were transiently cotransfected with constructs encoding N-terminal FLAG-tagged wild-type or mutant ITPKB together with green fluorescent protein (GFP). Immunoblotting revealed reduced expression and lower molecular weight of the mutant protein compared with wild-type ITPKB, with comparable expression of GFP (Fig 1, E). FLAG-tagged mutant and wild-type proteins were purified from transfected HEK293T cells and assessed

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

for their ability to phosphorylate IP3. The ITPKB^{Arg862Glufs*4} mutant completely lacked kinase activity (Fig 1, F). As a complementary approach for demonstrating the impact of kinase-dead ITPKB, we treated normal human T cells with BAMB-4, a known inhibitor of ITPKB kinase.[6] BAMB-4 inhibited Itk phosphorylation, promoted pAKT phosphorylation, and inhibited T-cell proliferation following CD3/CD28 ligation without affecting ITKPB expression (Fig 1, G–I). These findings provide further evidence that the absent kinase activity of the ITPKB^{Arg862Glufs*4} mutant underlies the T-cell deficiency in the patient.

Itpkb$^{-/-}$ mice are normal at birth, but grow poorly and die by age 6 months, and have a cell-intrinsic, severe block in thymocyte differentiation from the CD4^{+}CD8^{+} stage to the single positive stage, resulting in profoundly decreased CD4^{+} and CD8^{+} cell numbers in thymus and spleen.[4,5] B-cell development is normal, but splenic B cells are reduced by 60% to 80% and respond poorly to antigen receptor activation.[3] IgG, but not IgM, levels are reduced. *Itpbk*$^{-/-}$ mice develop progressive bone marrow failure with anemia, neutropenia, and thrombocytopenia, related in part to overactive AKT/mTOR signaling due to the loss of the restraint IP4 imposes on AKT activation by PIP3.[7] Sepsis, overwhelming infection, and the effect of the mutation on progenitor cells may have contributed to the anemia, neutropenia, and thrombocytopenia in the patient. The similarities between the patient and the *Itpbk*$^{-/-}$ mice (Table I), in conjunction with absence of ITPKB^{Arg862Glufs*4} kinase activity, support a pathogenic role of the patient's *ITPKB* mutation. Unlike *Itpbk*$^{-/-}$ mice, the patient had normal B-cell number, a difference also noted in IL-7RA deficiency.[8]

ITPKB haploinsufficiency due to microdeletion of chromosome 1q42.1-q42.3 was reported in an adult with common variable immunodeficiency.[9] In addition, the expressed ITPKB allele contained 2 polymorphisms. ITPKB expression was decreased by approximately 50%. The numbers of T and B cells were decreased, unlike in our patient's parents.

We report the first patient with T^{-}B^{+}NK^{+} severe combined immunodeficiency with a kinase-dead ITPKB mutant, expanding the molecular defects associated. Our findings underscore the importance of considering ITPKB deficiency in newborns with severe T-cell lymphopenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by the Perkin Foundation.

REFERENCES

1. Elich M, Sauer K. Regulation of hematopoietic cell development and function through phosphoinositides. Front Immunol 2018;9:931. [PubMed: 29780388]
2. Mukherjee S, Rigaud S, Seok SC, Fu G, Prochenka A, Dworkin M, et al. In silico modeling of Itk activation kinetics in thymocytes suggests competing positive and negative IP4 mediated feedbacks increase robustness. PLoS One 2013;8:e73937. [PubMed: 24066087]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3. Miller AT, Sandberg M, Huang YH, Young M, Sutton S, Sauer K, et al. Production of Ins(1,3,4,5)P4 mediated by the kinase Itpkb inhibits store-operated calcium channels and regulates B cell selection and activation. Nat Immunol 2007;8:514–21. [PubMed: 17417640]
4. Pouillon V, Hascakova-Bartova R, Pajak B, Adam E, Bex F, Dewaste V, et al. Inositol 1,3,4,5-tetrakisphosphate is essential for T lymphocyte development. Nat Immunol 2003;4:1136–43. [PubMed: 14517551]
5. Wen BG, Pletcher MT, Warashina M, Choe SH, Ziaee N, Wiltshire T, et al. Inositol (1,4,5) trisphosphate 3 kinase B controls positive selection of T cells and modulates Erk activity. Proc Natl Acad Sci U S A 2004;101:5604–9. [PubMed: 15064401]
6. Pan C, Jin L, Wang X, Li Y, Chun J, Boese AC, et al. Inositol-triphosphate 3-kinase B confers cisplatin resistance by regulating NOX4-dependent redox balance. J Clin Invest 2019;129:2431–45. [PubMed: 31081803]
7. Siegemund S, Rigaud S, Conche C, Broaten B, Schaffer L, Westernberg L, et al. IP3 3-kinase B controls hematopoietic stem cell homeostasis and prevents lethal hematopoietic failure in mice. Blood 2015;125:2786–97. [PubMed: 25788703]
8. Giliani S, Mori L, de Saint Basile G, Le Deist F, Rodriguez-Perez C, Forino C, et al. Interleukin-7 receptor alpha (IL-7Ralpha) deficiency: cellular and molecular bases. Analysis of clinical, immunological, and molecular features in 16 novel patients. Immunol Rev 2005;203:110–26. [PubMed: 15661025]
9. Louis AG, Yel L, Cao JN, Agrawal S, Gupta S. Common variable immunodeficiency associated with microdeletion of chromosome 1q42.1-q42.3 and inositol 1,4,5-trisphosphate kinase B (ITPKB) deficiency. Clin Transl Immunol 2016;5:e59.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

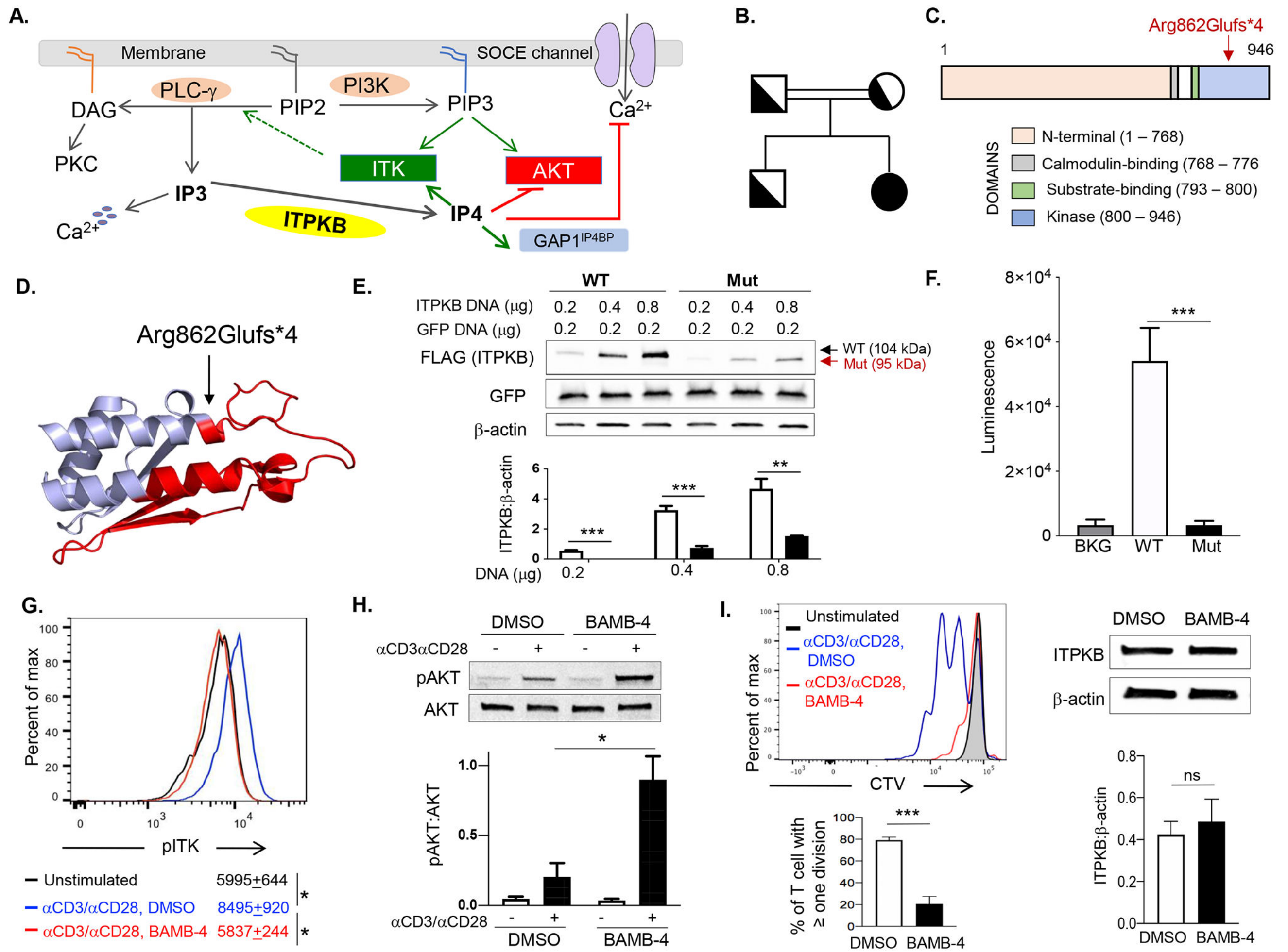

FIG 1.

Homozygous ITPKB mutation in the patient. **A**, Schematic diagram of the role of ITPKB in cell signaling. **B**, Family pedigree. **C**, Linear map of ITPKB with the patient's mutation in red. **D**, Ribbon diagram of the ITPKB kinase domain, with the fragment truncated in the patient's ITPKB$^{p.Arg862Glufs*4}$ mutant depicted in red. **E**, Top: Immunoblotting for FLAG, GFP, and actin of lysates from HEK293T cells cotransfected with N-terminal Flag-tagged WT or mutant ITPKB together with GFP. Bottom: Quantification of the data from 5 independent experiments. **F**, ITPKB kinase activity assessed using ADP-Glo with IP3 substrate. Luminescence normalized to protein input. *BKG*, Background; *Mut*, mutant; *WT*, wild-type. n = 3 independent experiments, **G-I**, Effect of the ITPKB inhibitor BAMB-4 on the activation of normal T cells by anti-CD3^{+}anti-CD28 as assessed by ITK phosphorylation after 2 minutes examined by intracellular FACS analysis (Fig 1, G), AKT phosphorylation after 20 minutes examined by immunoblotting (Fig 1, H), and proliferation examined by CTV dilution and ITPKB expression after 72 hours (Fig 1, I). Data in each panel show a representative experiment and the quantitative analysis of 3 independent experiments. *DMSO*, Dimethyl sulfoxide. Values and columns and bars in Fig 1, E–I, represent mean ± SD. *$P < .05$, **$P < .01$, ***$P < .001$.

TABLE I.

Comparison of the findings in the patient and Itpkb$^{-/-}$ mice

Finding	Patient (ITPKB^{Arg862Glufs*})		*Itpkb*$^{-/-}$ mice
Clinical phenotype	Failure to thrive Oral thrush Recurrent pneumonia Skin abscesses Dead at 11 mo		Poor growth Partial hair loss Giardia colitis All dead by age 6 mo
Lymphocytes			
CD4$^+$ T cells	*81* cells/μL	(1400–5100)	*2%* of LN cells (35%–65%)
CD8$^+$ T cells	*4* cells/μL	(600–2100)	*1.5%* of LN cells (19%–30%)
B cells	*534* cells/μL	(500–2500)	*12* $\times$ *10^6*/spleen (43×10^6–77×10^6)
Serum immunoglobulins			
IgG	*215* mg/dL	(215–704)	*9%* of WT control
IgA	60 mg/dL	(8–68)	*30%* of WT control
IgM	65 mg/dL	(35–105)	160% of WT control
Red cell mass	*7* g/dL	(11.3–14.1)	*7* $\times$ *10^6* cells/mm^3 (9×10^6–1×10^7)

LN, Lymph node; *WT*, wild-type.

Values in parentheses represent the normal range.

Values in italics represent abnormal values.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript