American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Absent B cells, agammaglobulinemia, and hypertrophic cardiomyopathy in Folliculin Interacting Protein 1 deficiency

Tracking no: BLD-2020-006441R2

Francesco Saettini (Fondazione MBBM, University of Milan-Bicocca, Italy) Cecilia Poli (3Facultad de Medicina Clínica Alemana de Santiago, Universidad del Desarrollo, Santiago, Chile) Jaime Vengoechea (Department of Medicine, Emory University, Atlanta, United States) Sonia Bonanomi (Fondazione MBBM, Italy) Julio Orellana (Hospital de Niños de la Santísima Trinidad, Argentina) Grazia Fazio (University of Milan Bicocca, Italy) Fred Rodriguez (Emory University, United States) Loreani Noguera (Universidad del Desarrollo, Chile) Claire Booth (UCL GOSH Institute of Child Health, United Kingdom) Valentina Jarur-Chamy (Universidad del Desarrollo, Chile) Marissa Shams (Emory University, United States) Maria Iascone (Papa Giovanni XXIII Hospital, Italy) Maja Vukic (Leiden University Medical Center, Netherlands) Serena Gasperini (Fondazione MBBM, University of Milano Bicocca, Italy) Manuel Quadri (University of Milan Bicocca, Italy) Amairelys Belen Barroeta Seijas (University College London, United Kingdom) Elizabeth Rivers (UCL Great Ormond Street Institute of Child Health, United Kingdom) Mario Mauri (University of Milano Bicocca, Italy) Raffaele Badolato (University of Brescia, Italy) Giovanni Cazzaniga (Centro Ricerca Tettamanti, University of Milan Bicocca, Italy) Cristina Bugarin (University of Milan Bicocca, Italy) Giuseppe Gaipa (Tettamanti Research center, Italy) Wilma Kroes (Leiden University Medical Center, Netherlands) Daniele Moratto (Flow Cytometry Unit, Clinical Chemistry Laboratory, ASST Spedali Civili di Brescia, Italy) Monique van Ostaijen-ten Dam (LUMC, Netherlands) Frank Baas (Leiden University Medical Center, Netherlands) Silvere van der Maarel (Leiden University Medical Center, Netherlands) Rocco Piazza (University of Milano - Bicocca, Italy) Zeynep Coban-Akdemir (Baylor Genetics Laboratory, Houston, TX, United States) James Lupski (Baylor-Hopkins Center for Mendelian Genomics, Houston, United States) Bo Yuan (Baylor College of Medicine Department of Molecular and Human Genetics, United States) Ivan Chinn (Section of Immunology, Allergy, and Rheumatology, Texas Children's Hospital, Houston, TX, United States) Lucia Daxinger (Leiden University Medical Center, Netherlands) Andrea Biondi (Pediatric Hematology Department, Fondazione MBBM, University of Milan-Bicocca, Italy)

Abstract:
Agammaglobulinemia is the most profound primary antibody deficiency that can occur due to an early termination of B-cell development. We here investigated three novel patients, including the first known adult, from unrelated families with agammaglobulinemia, recurrent infections, and hypertrophic cardiomyopathy (HCM). Two of them also presented with intermittent or severe chronic neutropenia. We identified homozygous or compound heterozygous variants in the gene for Folliculin interacting protein 1 (*FNIP1*), leading to loss of the FNIP1 protein. B-cell metabolism, including mitochondrial numbers and activity and PI3K/AKT pathway, was impaired. These defects recapitulated the *Fnip1*$^{-/-}$ animal model. Moreover, we identified either uniparental disomy or copy number variants [CNV] in two patients, expanding the variant spectrum of this novel inborn error of immunity. The results indicate that FNIP1 deficiency can be caused by complex genetic mechanisms and support the clinical utility of exome sequencing and CNV analysis in patients with broad phenotypes, including agammaglobulinemia and HCM.

FNIP1 deficiency is a novel inborn error of immunity characterized by early and severe B-cell development defect, agammaglobulinemia, variable neutropenia, and HCM. Our findings elucidate a functional and relevant role of FNIP1 in B-cell development and metabolism and potentially neutrophil activity.

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: Contributions: F.S., M.C.P., L.D., C.B., M.I. designed and analyzed experiments; L.P.N, V.J.C, A.B., G.F., M.Q., C.B., M.V., W.K., M.O performed experiments; J.C.O., S.B., S.G. provided samples and interpreted clinical and/or imaging data; J.R.L.,S.V.D.M, F.B., R.P., A.B., R.B., G.C. were involved in study design, gave intellectual input and critically revised the manuscript; D.M. conducted immunophenotyping on BM and PBMC; F.S, M.C.P, I.C., J.V., F.R., and M.S. wrote the manuscript which was approved by all authors. The authors declare no competing financial interests.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Data will be shared if requested to the corresponding author by email

Clinical trial registration information (if any):

Absent B cells, agammaglobulinemia, and hypertrophic cardiomyopathy in Folliculin Interacting Protein 1 deficiency

Francesco Saettini,[1] Cecilia Poli,[2,3] Jaime Vengoechea,[4,5] Sonia Bonanomi,[1] Julio C. Orellana,[6] Grazia Fazio,[7] Fred H. Rodriguez III,[8] Loreani P. Noguera,[3] Claire Booth,[9] Valentina Jarur-Chamy,[3] Marissa Shams,[5] Maria Iascone,[10] Maja Vukic,[11] Serena Gasperini,[12] Manuel Quadri,[7] Amairelys Barroeta Seijas,[9] Elizabeth Rivers,[9] Mario Mauri,[13] Raffaele Badolato,[14] Gianni Cazzaniga,[7,13] Cristina Bugarin,[7] Giuseppe Gaipa,[7] Wilma G.M. Kroes,[15] Daniele Moratto,[16] Monique M. van Oostaijen-ten Dam,[17] Frank Baas,[15] Silvère van der Maarel,[11] Rocco Piazza,[13] Zeynep H. Coban-Akdemir,[18,19] James R. Lupski,[18,20] Bo Yuan,[18,19] Ivan K. Chinn,[2,21,*] Lucia Clemens-Daxinger,[11,*] and Andrea Biondi,[1,7,*]

[1]Pediatric Hematology Department, Fondazione MBBM, University of Milano Bicocca, Monza, Italy. [2]Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030. [3]Facultad de Medicina Clínica Alemana de Santiago, Universidad del Desarrollo, Santiago, RM 7590943, Chile. [4]Department of Human Genetics, Emory University, Atlanta, GA 30322. [5]Department of Medicine, Emory University, Atlanta, GA 30322. [6]Division Alergia e Inmunología Clínica, Hospital de Niños de la Santísima Trinidad, Córdoba. Argentina. [7]Centro Ricerca Tettamanti, University of Milano Bicocca, Monza, Italy. [8]Departments of Medicine and Pediatrics, Sections of Cardiology, Emory University, Atlanta, GA 30322. [9]Molecular and Cellular Immunology Section, UCL Institute of Child Health, London, UK. [10]Molecular Genetics Laboratory, USSD LGM, Papa Giovanni XXIII Hospital, Bergamo, Italy. [11]Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands. [12]Metabolic Rare Disease Unit, Pediatric Department, Fondazione MBBM, University of Milano Bicocca, Monza, Italy. [13]Department of Medicine and Surgery, University of Milano Bicocca and San Gerardo hospital, 20900, Monza, Italy. [14]Pediatrics Clinic and Institute of Molecular Medicine A. Novicelli, Department of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili of Brescia, Brescia, Italy. [15]Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands. [16]Flow cytometry Laboratory, Diagnostic Department, ASST Spedali Civili di Brescia, Brescia, Italy. [17]Department of Pediatrics, Leiden University Medical Center Leiden, Netherlands. [18]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030. [19]Baylor Genetics Laboratory, Houston, TX 77030. [20]Baylor-Hopkins Center for Mendelian Genomics, Houston, TX 77030. [21]Section of Immunology, Allergy, and Rheumatology, Texas Children's Hospital, Houston, TX 77030.

*IKC, LD, and AB contributed equally to this study.

Running head

Agammaglobulinemia and HCM in FNIP1 deficiency

Correspondig author

Francesco Saettini, Pediatric Hematology Department, Fondazione MBBM, University of Milano Bicocca, Via Cadore, 20900, Monza, Italy. Phone +390392333529. Fax: +390392332470. Mail: francescosaettini@yahoo.it

Absent B cells, agammaglobulinemia, and hypertrophic cardiomyopathy in Folliculin Interacting Protein 1 deficiency

Francesco Saettini,[1] Cecilia Poli,[2,3] Jaime Vengoechea,[4,5] Sonia Bonanomi,[1] Julio C. Orellana,[6] Grazia Fazio,[7] Fred H. Rodriguez III,[8] Loreani P. Noguera,[3] Claire Booth,[9] Valentina Jarur-Chamy,[3] Marissa Shams,[5] Maria Iascone,[10] Maja Vukic,[11] Serena Gasperini,[12] Manuel Quadri,[7] Amairelys Barroeta Seijas,[9] Elizabeth Rivers,[9] Mario Mauri,[13] Raffaele Badolato,[14] Gianni Cazzaniga,[7,13] Cristina Bugarin,[7] Giuseppe Gaipa,[7] Wilma G.M. Kroes,[15] Daniele Moratto,[16] Monique M. van Oostaijen-ten Dam,[17] Frank Baas,[15] Silvere van der Maarel,[11] Rocco Piazza,[13] Zeynep H. Coban-Akdemir,[18,19] James R. Lupski,[18,20] Bo Yuan,[18,19] Ivan K. Chinn,[2,21,*] Lucia Clemens-Daxinger,[11,*] and Andrea Biondi,[1,7,*]

Key Points

FNIP1 deficiency causes agammaglobulinemia, variable neutropenia, and hypertrophic cardiomyopathy.

FNIP1 deficiency alters B-cell development and metabolism.

Key words

Agammaglobulinemia; neutropenia; FNIP1, inborn error of immunity; primary immunodeficiency; hypertrophic cardiomyopathy.

Abstract

Agammaglobulinemia is the most profound primary antibody deficiency that can occur due to an early termination of B-cell development. We here investigated three novel patients, including the first known adult, from unrelated families with agammaglobulinemia, recurrent infections, and hypertrophic cardiomyopathy (HCM). Two of them also presented with intermittent or severe chronic neutropenia. We identified homozygous or compound heterozygous variants in the gene for Folliculin interacting protein 1 (*FNIP1*), leading to loss of the FNIP1 protein. B-cell metabolism, including mitochondrial numbers and activity and PI3K/AKT pathway, was impaired. These defects recapitulated the *Fnip1*$^{-/-}$ animal model. Moreover, we identified either uniparental disomy or copy number variants [CNV] in two patients, expanding the variant spectrum of this novel inborn error of immunity. The results indicate that FNIP1 deficiency can be caused by complex genetic mechanisms and support the clinical utility of exome sequencing and CNV analysis in patients with broad phenotypes, including agammaglobulinemia and HCM.

FNIP1 deficiency is a novel inborn error of immunity characterized by early and severe B-cell development defect, agammaglobulinemia, variable neutropenia, and HCM. Our findings elucidate a functional and relevant role of FNIP1 in B-cell development and metabolism and potentially neutrophil activity.

Introduction

Agammaglobulinemia is the most profound primary antibody deficiency that results from early termination of B-cell development, which leads to the absence of mature circulating B-cells and very low or absent serum immunoglobulin levels. To date, defects in *BTK*, *IGHM*, *IGLL1*, *CD79A*, *CD79B*, *BLNK*, and *PIK3R1* have been reported to cause agammaglobulinemia[1].

Disruption of Folliculin Interacting Protein 1 (FNIP1) alters the essential metabolic regulators AMPK and mTOR (Figure 1A), resulting in profound B-cell deficiency and decreased NKT cells, hypertrophic cardiomyopathy (HCM), and pre-excitation syndrome[2-8]. Contemporarily, another group[9] clinically described two families with inborn FNIP1 deficiency with hypogammaglobulinemia, intermittent neutropenia, and HCM. Here, we present functional validation of FNIP1 deficiency in three novel families including the first adult case. FNIP1 deficiency results in HCM, absent circulating B-cells, agammaglobulinemia, and either severe or intermittent neutropenia.

Methods

This study was approved by the institutional review boards/ethic committee of Comitato Etico Brianza (Monza, Italy; PID-GENMET), and Baylor College of Medicine (Houston, Texas). All study participants provided written informed consent.

Full methods are detailed in the supplementary data.

Results and discussion

Clinical phenotype

P1 was born to consanguineous parents. The parents of P2 and P3 denied consanguinity (Figure 1B). Clinical manifestations started in infancy (< 1 year) including severe and/or recurrent infections (detailed clinical histories in the supplementary data). Sinopulmonary infections led to bronchial wall thickening (P1), extensive bronchiectasis requiring lobectomy (P2), or calcifications (P3; supplemental Figure 1). All patients had left ventricular HCM (supplemental Figure 2 and supplemental Table 1). P2 also had an interatrial communication requiring surgical correction, and P3 had severe tricuspid valve regurgitation, severe right ventricle dilatation, and pre-excitation syndrome. All patients had no imaging or laboratory signs of renal disease. For P1, neurological examination showed developmental delay associated with MRI abnormalities (supplemental Figure 3). P3 had Crohn disease that required multiple bowel surgeries. All patients had absent circulating B-cells and agammaglobulinemia (Table 1) requiring immunoglobulin replacement therapy. Two out of three patients had neutropenia, either severe (P1; neutrophil count consistently below 0.5 $x10^9$/l) or intermittent (P3), which was confirmed outside the infectious episodes and may have contributed to recurrent and severe infections.

FNIP1 variants in patients with agammaglobulinemia

Trio whole-exome sequencing (WES) was performed in all families. No candidates were identified within recognized PID-associated genes[10-13]. We identified distinct biallelic variants in *FNIP1*, which were not present in public databases (gnomAD, ESP, and 1000 Genomes) and exclusive to these families in our internal databases. For P1, the homozygous NM_133372.2:c.868C>T nonsense variant in *FNIP1* is located in exon 9 and predicted to result in a premature stop codon (p.R290*). The variant was confirmed by Sanger sequencing and each parent was a heterozygous carrier (Figure 1B; supplemental figure 4A). A homozygous splicing donor variant (c.3306+1G>A; supplemental Figure 5) was identified in P2. Sanger sequencing confirmed the father to be a heterozygous carrier of the variant whilst it was not present in the mother. Exome data were consistent with paternal uniparental disomy (UPD) of chromosome 5 leading to homozygosity of this variant in P2 (supplemental methods and supplemental Figure 6). For P3, WES showed a maternally inherited deletion of *FNIP1* exons 9-18 and a paternally inherited single nucleotide variant (SNV; c.3218delT;p.L1073Wfs*32; Figure 1B).

We evaluated functional consequences of the *FNIP1* variants in the blood samples. *FNIP1* mRNA levels were significantly decreased in P1 but not absent, most likely due to incomplete nonsense-mediated decay

(Figure 1C). Because FNIP1 is expressed in activated peripheral lymphocytes[3,5], to determine whether the variants altered protein stability, we examined the presence of FNIP1 protein in stimulated cultured T-cells. Immunoblotting demonstrated the complete absence of FNIP1 in all the patients (Figure 1D).

Immune-cell phenotype in FNIP1 deficiency

Analysis of the T-cell subsets showed mildly and intermittently increased $CD3^+$ in P1 and P3. Standard lymphocyte proliferative response to specific antigens and mitogens was normal in P1 and P3 and decreased in P2 (Table 1). Using IL-2 and anti-CD28/CD3, P1 T-cells showed increased apoptosis between days 7 to 11. Apoptosis was more prominent for $CD8^+$ compared to $CD4^+$ T-cells (supplemental Figure 7). NKT cells were decreased in P1 Table 1).

Peripheral B-lymphocytes were undetectable or markedly decreased in all patients (Table 1). For P1, bone marrow examination displayed delayed granulocyte maturation, with no overt arrest. B-cell precursors did not show evidence of a maturation block, unlike classical agammaglobulinemia[14], although a relative increase of earlier maturation stages (pro-B and pre-B1) and a significant reduction of immature B-cells was observed (Figure 1E and supplemental Figure 8).

FNIP1 deficiency is associated with altered cell metabolism

We hypothesized that human FNIP1 deficiency may hamper B-cell metabolism similar to Fnip1-deficient mice[2-8]. Indeed, circulating P1 B-cells exhibited increased numbers of mitochondria and mitochondrial activity relative to healthy control (HC) B-cells (Figure 1F and supplemental Figure 9). Next, we examined the activation of the PI3K/Akt pathway. We observed constitutive hyperactivation of PI3K downstream targets in P1 B-cell progenitors relative to HC B-cell precursors (Figure 1G). Specifically, p4EBP1 and pAkt473 were significantly activated (12.46±1.86 vs 4.00±0.53, p = 0.0002; 3.82±1.78 vs 0.97±0.09, p = 0.029). As opposite, no difference could be found in S6 phosphorylation (1.46±0.14 vs 2.70±0.51, p = 0.14). To assess the accuracy of our approach[15], we tested NPV-BEZ 235 as a specific inhibitor of the PI3K pathway and observed that it was able to significantly decrease p4EBP1 (mean 12.46±1.86 vs 2.58±0.32, p = 0.006). These results suggest that the sensitivity to PI3K inhibition is determined by the activation of the PI3K/Akt pathway in FNIP1-deficient patients.

Mouse Fnip1 consists of 1,165 amino acids and shares 91% amino acid identity with human FNIP1[5]. Fnip1 plays a nonredundant role in early B-cell development and metabolism, skeletal muscle fiber type specification, and cardiac function[2-8]. Our report confirms that the clinical and immunological phenotypes are strikingly overlapping (i.e., HCM, pre-excitation syndrome, and early and severe B-cell defect with agammaglobulinemia). FNIP1 deficiency can be detected even in young adults. Moreover, we identified unconventional heterogeneous genetic etiologies for FNIP1 deficiency in two patients, expanding the variant spectrum of this novel inborn error of immunity (IEI; supplemental Figure 10A). In fact, only a limited number of cases of chromosome 5 UPD have been reported[16]. Our findings argue for the clinical use of exome sequencing with copy number variants analysis in patients with complex phenotypes. Importantly, in one patient we have provided for the first time some of the functional studies (increased number of mitochondria and mitochondrial activity and 4EBP1 activation) that recapitulate the alterations described in Fnip1 deficient mice.

Some aspects nonetheless remain controversial. Contradictory results concerning the activation of the PI3K/Akt pathway have been described in *Fnip1*$^{-/-}$ mice[7,17] (supplemental Table 2). Unlike Fnip1-deficient mice[17], no FNIP1 patients have had overt clinical symptoms of renal disease[9], but the presence of renal cysts has not been investigated by renal biopsies. Only one proband has had myopathy[9] (supplemental

Figure 10B). Here we report the coexistence of heart defects other than HCM and pre-excitation syndrome in FNIP1 deficiency. Elsewhere[9], patients carrying biallelic missense variants showed milder B-cell lymphopenia, and one individual had only decreased IgM with normal IgG and elevated IgA levels. These elements suggest that FNIP1 deficiency could be a protean disorder resembling also common variable immunodeficiency phenotype. The T-cell compartment was unaffected in *Fnip1*$^{-/-}$mice[5,7]. Although patients have not shown any clinical signs of defective T-cell function, lymphocytosis and/or defective T-cell proliferation with increased apoptosis have been found in some patients (Figure 10B). Granulocytes are not affected in the *Fnip1*$^{-/-}$ mouse[7], yet four patients have displayed severe or intermittent neutropenia, and two have monocytosis. This finding is reminiscent of the neutrophil survival defect seen in BTK-associated agammaglobulinemia[18]. As in other IEI[19,20], FNIP1 deficiency may be associated with severe or intermittent neutropenia, or even episodic normal neutrophil counts. Central nervous system involvement is present in four out of six patients. These manifestations only occurred in consanguineous pedigrees and may result either from other genetic changes not yet discovered or from FNIP1 phenotypic heterogeneity.

Collectively, we identified novel inherited *FNIP1* variants causing FNIP1 deficiency, which affects B-cell survival and metabolism recapitulating the Fnip1$^{-/-}$ animal model. FNIP1 deficiency should be considered in patients with hypo- or agammaglobulinemia, congenital heart defects, particularly HCM, and neutropenia.

Acknowledgements

The authors thank the patients and their families for participating in the study; Donna M. Muzny, Shalini N. Jhangiani, Richard A. Gibbs, and Haowei Du from the Baylor College of Medicine Human Genome Sequencing Center and Baylor-Hopkins Center for Mendelian Genomics for whole exome sequencing and bioinformatics support; Nicholas L. Rider and Gina Cahill from the Texas Children's Hospital William T. Shearer Center for Human Immunobiology and Jeffrey Modell Foundation at Texas Children's Hospital for validation studies; Francesco Canonico from Department of Neuroradiology, University of Milan-Bicocca, San Gerardo Hospital, ASST di Monza, Monza for brain CT study; Francesca Pluchinotta and Antonella Camporeale from IRCCS Policlinico San Donato, Milan, for cardiac MRI study; Giuseppe Limoli from USS Patologia Neonatale e Nido e Servizio di Cardiologia Pediatrica, ASST Lodi for cardiac ultrasound study; and GeneDx and GeneMatcher for facilitating collaboration.

This work was supported by NIH-NHGRI/NHLBI UM1 HG006542 (to the Baylor-Hopkins Center for Mendelian Genomics), Fondecyt #11181222 (to MCP), AIRC-IG Grant 22082 (to RP), "PG23/FROM 2017 Call for Independent Research" as part of the RARE project—Rapid Analysis for Rapid care (to Papa Giovanni XXIII), GOSH NIHR BRC, and Wellcome Trust Research grants (201250/Z/16/Z and 104807/Z14/Z to ER and AB, respectively).

Authorship contributions

Contributions: F.S., M.C.P., L.D., C.B., M.I. designed and analyzed experiments; L.P.N, V.J.C, A.B., G.F., M.Q., C.B., M.V., W.K., M.O performed experiments; J.C.O., S.B., S.G. provided samples and interpreted clinical and/or imaging data; J.R.L.,S.V.D.M, F.B., R.P., A.B., R.B., G.C. were involved in study design, gave intellectual input and critically revised the manuscript; D.M. conducted immunophenotyping on BM and PBMC; F.S, M.C.P, I.C., J.V., F.R., and M.S. wrote the manuscript which was approved by all authors.

Disclosure of conflict of interest

The authors declare no competing financial interests.

References

1. Ben-Ali M, Kechout N, Mekki N, et al. Genetic Approaches for Definitive Diagnosis of Agammaglobulinemia in Consanguineous Families. *J Clin Immunol.* 2020;40(1):96-104.

2. Baba M, Hong SB, Nirmala Sharma N, et al. Folliculin encoded by the BHD gene interacts with a binding protein, FNIP1, and AMPK, and is involved in AMPK and mTOR signaling. *Proc Nat Acad Sci USA.* 2006;103(42):15552-15557.

3. Park H, Staehling K, Tsang M, et al. Disruption of Fnip1 Reveals a Metabolic Checkpoint Controlling B Lymphocyte Development. *Immunity.* 2012;36(5):769-81.

4. Reyes NL, Banks GB, Tsang M, et al. Fnip1 Regulates Skeletal Muscle Fiber Type Specification, Fatigue Resistance, and Susceptibility to Muscular Dystrophy. *Proc Nat Acad Sci USA.* 2015;112(2):424-9.

5. Park H, Tsang M, Iritani BM, et al. Metabolic Regulator Fnip1 Is Crucial for iNKT Lymphocyte Development. *Proc Nat Acad Sci USA*. 2014;111(19):7066-71.

6. Siggs OM, Stockenhuber A, Deobagkar-Lele M, et al. Mutation of Fnip1 Is Associated With B-cell Deficiency, Cardiomyopathy, and Elevated AMPK Activity. *Proc Nat Acad Sci USA*. 2016;113(26):E3706-15.

7. Baba M, Keller JR, Sun HW, et al. The folliculin-FNIP1 pathway deleted in human Birt-Hogg-Dubé syndrome is required for murine B-cell development. *Blood*. 2012;120(6):254–1261.

8. Ramírez JA, Iwata T, Park H, et al. Folliculin Interacting Protein 1 Maintains Metabolic Homeostasis during B Cell Development by Modulating AMPK, mTORC1, and TFE3. *J Immunol*. 2019;ji1900395.

9. Niehues T, Turul Özgür TT, Bickes M, et al. Mutations of the Gene FNIP1 Associated With a Syndromic Autosomal Recessive Immunodeficiency With Cardiomyopathy and Pre-Excitation Syndrome. *Eur J Immunol*. 2020 Mar 17[Online ahead of print]

10. Stray-Pedersen A, Sorte HS, Samarakoon P, et al. Primary immunodeficiency diseases: Genomic approaches delineate heterogeneous Mendelian disorders. *J Allergy Clin Immunol*. 2017;139(1):232–245.

11. Lupski JR, Gonzaga-Jauregui C, Yang Y, et al. Exome sequencing resolves apparent incidental findings and reveals further complexity of SH3TC2 variant alleles causing Charcot-Marie-Tooth neuropathy. *Genome Med*. 2013;5(6):57.

12. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–424.

13. Chinn IK, Chan AY, Chen K, et al. Diagnostic interpretation of genetic studies in patients with primary immunodeficiency diseases: A working group report of the Primary Immunodeficiency Diseases Committee of the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin. Immunol*. 2020;145(1):46–69.

14. Noordzij JG, De Bruin-Versteeg S, Marieke Comans-Bitter W, et al. Composition of precursor B-cell compartment in bone marrow from patients with X-linked agammaglobulinemia compared with healthy children. *Pediatr. Res*. 2002;51(2):159–168.

15. Gaipa G, Bugarin C, Longoni D, et al. Aberrant GM-CSF signal transduction pathway in juvenile myelomonocytic leukemia assayed by flow cytometric intracellular STAT5 phosphorylation measurement. *Leukemia.* 2009;23:791–793.

16. Kunwar F, Pabst R, Bakshi S. Intrauterine Growth Restriction Associated With Paternal Isodisomy of Chromosome 5: A Clinical Report and Literature Survey. *J Matern Fetal Neonatal Med.* 2020;33(6):1027-1029. doi: 10.1080/14767058.2018.1506443. Epub 2018 Sep 6.

17. Centini R, Tsang M, Iwata T, Park H, et al. (2018) Loss of Fnip1 alters kidney developmental transcriptional program and synergizes with TSC1 loss to promote mTORC1 activation and renal cyst formation. *PLoS ONE.* 2018;13(6): e0197973. https://doi.org/10.1371/journal.pone.0197973

18. Honda F, Kano H, Kanegane H, et al. The Kinase Btk Negatively Regulates the Production of Reactive Oxygen Species and Stimulation-Induced Apoptosis in Human Neutrophils. *Nat Immunol.* 2012;13(4):369-78.

19. Saettini F, Cattoni A, D'Angio' M, et al. Intermittent granulocyte maturation arrest, hypocellular bone marrow, and episodic normal neutrophil count can be associated with SRP54 mutations causing Shwachman-Diamond-like syndrome. *Br J Haematol*. 2020;Mar 20. doi: 10.1111/bjh.16585. Online ahead of print.

20. Pasquet M, Bellanné-Chantelot C, Tavitian S, et al. High frequency of GATA2 mutations in patients with mild chronic neutropenia evolving to MonoMac syndrome, myelodysplasia, and acute myeloid leukemia. *Blood*. 2013;121(5):822-829.

Figure 1. Functional studies on patients with FNIP1 deficiency. (A) Schematic illustration displaying the interaction of FNIP1 in B-cells. Positive and negative regulators of mTORC1 signaling are depicted in green and in blue, respectively. Folliculin and Fnip1/2 have been described as both positive and negative regulators of mTORC1. (B) Pedigrees showing three families with affected individuals harboring *FNIP1* variants. Solid symbols indicate affected persons who were homozygous or compound heterozygous for the mutant alleles; half solid symbols, heterozygous persons; circles, female family members; square, male family members; double lines, consanguinity. (C) Expression of FNIP1 mRNA in P1 (RT-qPCR analysis). Data are expressed as mean ± standard deviation (SD) (2 independent experiments, each one performed in triplicates). Statistical test analysis was performed using 1-way ANOVA. (D) FNIP1 protein expression in T-cells. (E) Bone marrow B-cell immunophenotyping in P1 compared with a healthy control and one BTK patient (representative experiment). (F) Quantification of total mitochondrial abundance and mitochondrial activity in circulating $CD19^+$ cells isolated from an healthy control and P1 (representative experiment). (G) Evaluation of pAKT, pS6, and p4EBP1 levels in B-cell bone marrow progenitors from P1 and a healthy control (2 experiments). In all graphs ** $p<0.01$, *** $p<0.001$. Means ± SD.

HC = healthy control.

Table 1. Immunological characteristics of patients with FNIP1 deficiency.

^Before G-CSF treatment. [§]Before or without Ig replacement therapy. N/A = not available.

	P1		P2		P3	
Age at the time of evaluation, years	3		9		25	
Coordinates (hg19) Chr5	Homozygous 131042150_G>A		Homozygous 130987494_C>T		Compuond heterozygous 130766131_131044371 (min interval)	130987583del
Variant(s) [NM_133372.2]	c.C868T		c.3306+1G>A		ex9-ex18del	c.3218delT
Protein change	p.290*		N/A		N/A	p.L1073Wfs*32
CADD score	36		34		N/A	36
		Age-matched normal values		**Age-matched normal values**		**Age-matched normal values**
Hemoglobin (g/dl)	9.7	11.5-13.5	12.3	11.5-14.5	11.8	12.0-16.0
WBC (10^3 cells/L)	9.04	5.2-11.0	20.14	5.2-11.0	3.8	4.4-8.1
Neutrophils (10^3 cells/L)	0.28^	>1.5	13.29	>1.5	0.76	>1.5
Lymphocytes (10^3 cells/L)	4.7	2.3-5.4	3.63	2.3-5.4	2.51	1.4-3.3
Monocytes (10^3 cells/L)	1.8^	<1.0	2.62	<1.0	0.53	<1.0
Platelets (10^3 cells/L)	423	>100	440	>100	253	>100
Lymphocyte subsets						
$CD3^+$ (10^3 cells/L)	4.23	1.4-3.7	3.35	1.4-3.7	3.03	0.6-2.2
$CD3^+CD4^+$ (10^3 cells/L)	3.09	0.7-2.2	1.80	0.7-2.2	1.42	0.4-1.4
$CD45RA^+CCR7^+CD31^+$ %	62.1	36.2-71.8	N/A	20.3-68.9	N/A	N/A
Naïve %	81.0	49.2-85.8	62.8	37.8-80.3	31.0	2.0-58.0
Effector memory %	3.9	2.8-16.9	5.25	4.0-25.5	10.0	0.7-12.0
Central memory %	14.9	9.6-31.9	31.69	9.9-41.1	39.0	6.0-47.0
Term.Diff. %	0.2	0.6-4.8	0.26	0.4-7.7	N/A	N/A
$CD3^+CD8^+$ (10^3 cells/L)	1.0	0.49-1.3	1.48	0.49-1.3	1.39	0.2-0.86
Naïve %	79.3	22.8-79.9	59.6	20.3-78.2	39.0	6.0-84.0
Central %	4.6	0.9-11.3	29.82	1.7-13.3	15.0	1.0-18.0
Effector memory %	6.7	4.3-31.4	4.99	8.6-34.5	5.0	0.0-6.0
Term.Diff %	9.4	6.8-52.7	5.58	7.0-53.8	N/A	N/A
$CD19^+$ (10^3 cells/L)	0.05	0.39-1.4	0.0	0.39-1.4	0.0	0.09-0.4
Recent B emigrants %	20.6	10.6-42.6	0.0	7.1-35.3	N/A	N/A
Naïve %	8.6	34.2-65.5	0.0	37.1-70.2	N/A	N/A
$CD19^{hi}CD21^{lo}$	68.2	1.5-9.8	0.0	1.9-9.0	N/A	N/A
Unswitched %	1.4	2.9-15.3	0.0	3.1-18.3	N/A	N/A
Switched %	0.0	1.5-14.2	0.0	2.4-19.8	N/A	N/A
Term.Diff	0.0	0.37-15.3	0.0	0.3-11.8	N/A	N/A
Plasma cells	0.0	0.06-4.1	0.0	0.1-2.7	N/A	N/A
NKT (10^3 cells/L)	0.005	0.015-0.25	0.057	0.012-0.34	0.5	0.015-0.34
NK (10^3 cells/L)	0.44	0.13-0.72	0.26	0.13-0.72	0.17	0.1-0.5
DC, plasmacytoid %	0.16	0.16-0.76	N/A	N/A	N/A	N/A
DC, myeloid %	0.23	0.18-0.92	N/A	N/A	N/A	N/A
Immunoglobulins (g/l)[§]						
IgG	0.13	4.9-16.1	0.33	5.4-16.1	3.83	6.0-16.0
IgA	<0.01	0.4-2.0	<0.01	0.7-2.5	<0.02	0.8-2.8
IgM	0.08	0.5-2.0	0.14	0.5-1.8	<0.1	0.5-1.9
IgE kU/l	<1	<33	<1	<48	<1	<48
Tetanus vaccine titer	Absent[§]		N/A		N/A	

T-cell proliferation					
CD3	Normal		N/A		N/A
CD3 + IL2	Normal		N/A		N/A
PHA	Normal		N/A		Normal
Tetanus	N/A		N/A		Normal
Candida	N/A		N/A		Normal
Con A	N/A		N/A		Normal
CD3 + CD2 + CD8 + IL-2	N/A		Reduced		N/A