



**Universiteit
Leiden**
The Netherlands

Absent B cells, agammaglobulinemia, and hypertrophic cardiomyopathy in folliculin-interacting protein 1 deficiency

Saettini, F.; Poli, C.; Vengoechea, J.; Bonanomi, S.; Orellana, J.C.; Fazio, G.; ... ; Biondi, A.

Citation

Saettini, F., Poli, C., Vengoechea, J., Bonanomi, S., Orellana, J. C., Fazio, G., ... Biondi, A. (2021). Absent B cells, agammaglobulinemia, and hypertrophic cardiomyopathy in folliculin-interacting protein 1 deficiency. *Blood*, 137(4), 493-499.
doi:10.1182/blood.2020006441

Version: Publisher's Version
License: [Leiden University Non-exclusive license](#)
Downloaded from: <https://hdl.handle.net/1887/3209343>

Note: To cite this publication please use the final published version (if applicable).

IMMUNOBIOLOGY AND IMMUNOTHERAPY

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,^{8,9} Loreani P. Noguera,³ Claire Booth,¹⁰ Valentina Jarur-Chamy,³ Marissa Shams,⁵ Maria Iascone,¹¹ Maja Vukic,¹² Serena Gasperini,¹³ Manuel Quadri,⁷ Amarelys Barroeta Seijas,¹⁰ Elizabeth Rivers,¹⁰ Mario Mauri,¹⁴ Raffaele Badolato,¹⁵ Gianni Cazzaniga,^{7,14} Cristina Bugarin,⁷ Giuseppe Gaipa,⁷ Wilma G. M. Kroes,¹⁶ Daniele Moratto,¹⁷ Monique M. van Oostaijen-ten Dam,¹⁸ Frank Baas,¹⁶ Silvere van der Maarel,¹² Rocco Piazza,¹⁴ Zeynep H. Coban-Akdemir,^{19,20} James R. Lupski,^{19,21} Bo Yuan,^{19,20} Ivan K. Chinn,^{2,22,*} Lucia Daxinger,^{12,*} and Andrea Biondi^{1,7,*}

¹Pediatric Hematology Department, Fondazione Monza e Brianza per il Bambino e la sua Mamma (MBBM), University of Milano Bicocca, Monza, Italy; ²Department of Pediatrics, Baylor College of Medicine, Houston, TX; ³Facultad de Medicina Clínica Alemana de Santiago, Universidad del Desarrollo, Santiago, Chile; ⁴Department of Human Genetics, Emory University, Atlanta, GA; ⁵Department of Medicine, Emory University, Atlanta, GA; ⁶Division Alergia e Immunología Clínica, Hospital de Niños de la Santísima Trinidad, Córdoba, Argentina; ⁷Centro Ricerca Tettamanti, University of Milano Bicocca, Monza, Italy; ⁸Section of Cardiology, Department of Medicine, and ⁹Section of Cardiology, Department of Pediatrics, Emory University, Atlanta, GA; ¹⁰Molecular and Cellular Immunology Section, UCL Institute of Child Health, London, United Kingdom; ¹¹Molecular Genetics Laboratory, Università Settore Scientifico-Disciplinare Laboratorio di Genetica Medica (USSD LGM), Papa Giovanni XXIII Hospital, Bergamo, Italy; ¹²Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; ¹³Metabolic Rare Disease Unit, Pediatric Department, Fondazione MBBM, University of Milano Bicocca, Monza, Italy; ¹⁴Department of Medicine and Surgery, University of Milano Bicocca-San Gerardo Hospital, Monza, Italy; ¹⁵Pediatrics Clinic and Institute of Molecular Medicine A. Novicelli, Department of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili di Brescia, Brescia, Italy; ¹⁶Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; ¹⁷Flow Cytometry Laboratory, Diagnostic Department, ASST Spedali Civili di Brescia, Brescia, Italy; ¹⁸Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands; ¹⁹Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; ²⁰Baylor Genetics Laboratory, Houston, TX; ²¹Baylor-Hopkins Center for Mendelian Genomics, Houston, TX; and ²²Section of Immunology, Allergy, and Rheumatology, Texas Children's Hospital, Houston, TX

KEY POINTS

- FNIP1 deficiency causes agammaglobulinemia, variable neutropenia, and hypertrophic cardiomyopathy.
- FNIP1 deficiency alters B-cell development and metabolism.

Agammaglobulinemia is the most profound primary antibody deficiency that can occur due to an early termination of B-cell development. We here investigated 3 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 phosphatidylinositol 3-kinase/AKT pathway, was impaired. These defects recapitulated the *Fnip1*^{-/-} animal model. Moreover, we identified either uniparental disomy or copy-number variants (CNVs) in 2 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. (*Blood*. 2021;137(4):493-499)

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 natural killer (NK) T cells, hypertrophic cardiomyopathy (HCM), and pre-excitation syndrome.²⁻⁸ Contemporarily, another group⁹ clinically described 2 families with inborn FNIP1 deficiency with hypogammaglobulinemia, intermittent neutropenia, and HCM. Here, we present functional validation of FNIP1 deficiency in

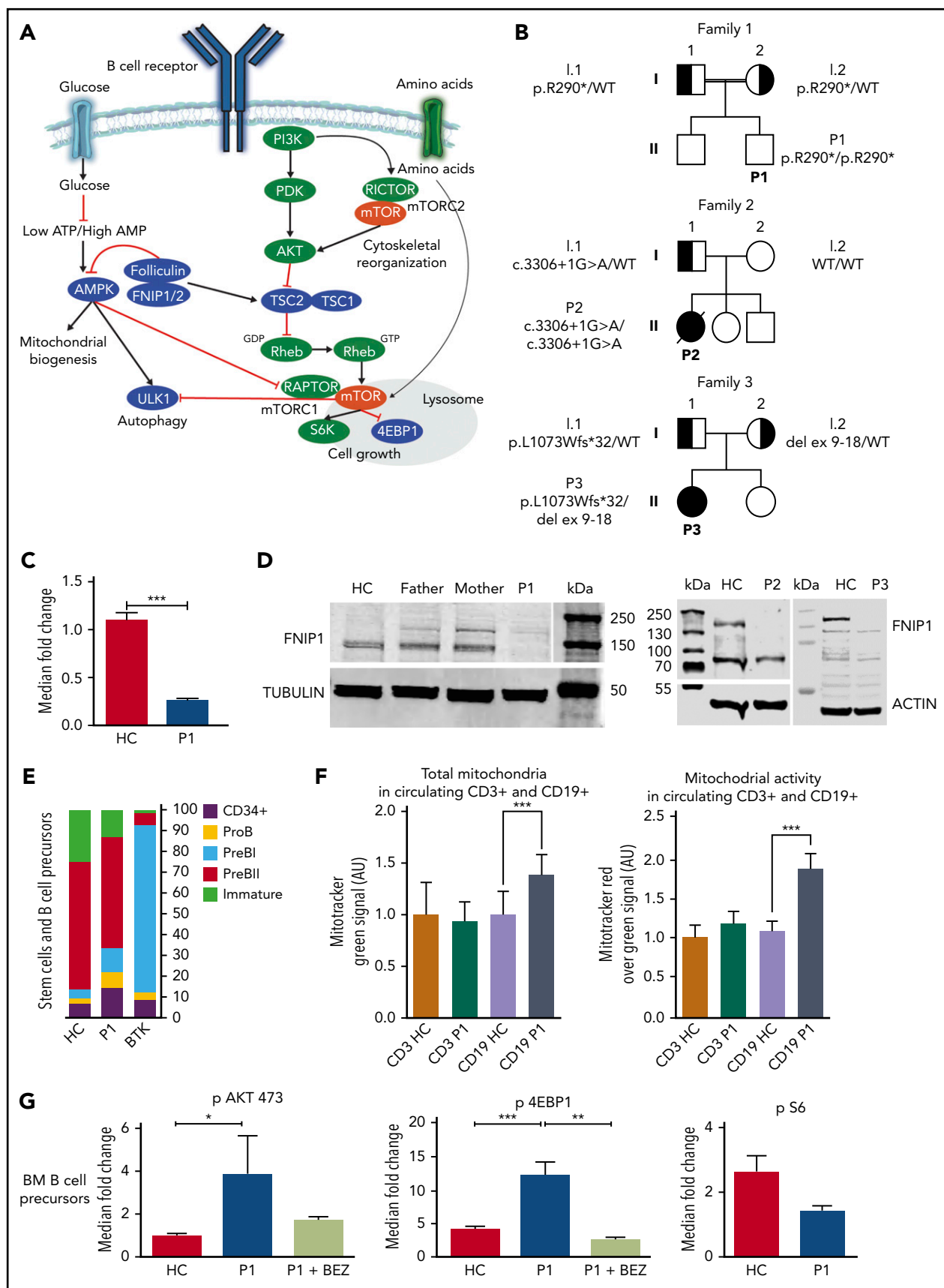


Figure 1. Functional studies on patients with FNIP1 deficiency. (A) Schematic illustration displaying the interaction of FNIP1 in B cells. Positive and negative regulators of mTORC1 signaling are depicted in green and in blue, respectively. Folliculin and Fip1/2 have been described as both positive and negative regulators of mTORC1. (B) Pedigrees showing 3 families with affected individuals harboring FNIP1 variants. Solid symbols indicate affected persons who were homozygous or compound heterozygous for

3 novel families, including the first adult case. *FNIP1* deficiency results in HCM, absent circulating B cells, agammaglobulinemia, and either severe or intermittent neutropenia.

Study design

This study was approved by the Institutional Review Boards/Ethics Committees of Comitato Etico Brianza (PID-GENMET; Monza, Italy) and Baylor College of Medicine (Houston, TX). All study participants provided written informed consent.

Full methods are detailed in supplemental Materials and methods (available on the *Blood* Web site).

Results and discussion

Clinical phenotype

Patient 1 (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; 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 magnetic resonance imaging 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 3 patients had neutropenia, either severe (P1; neutrophil count consistently $<0.5 \times 10^9/L$) 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 was performed in all families. No candidates were identified within recognized primary immunodeficiency-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, while it was not present in the mother. Exome data were consistent

with paternal uniparental disomy of chromosome 5 leading to homozygosity of this variant in P2 (supplemental Materials and methods; supplemental Figure 6). For P3, whole-exome sequencing showed a maternally inherited deletion of *FNIP1* exons 9 to 18 and a paternally inherited single-nucleotide variant (c.3218delT;p.L1073Wfs*32; Figure 1B).

We evaluated functional consequences of the *FNIP1* variants in the blood samples. *FNIP1* messenger RNA 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 and 11. Apoptosis was more prominent for CD8⁺ compared with CD4⁺ T cells (supplemental Figure 7). NK T 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 were observed (Figure 1E; 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 B cells (Figure 1F; supplemental Figure 9). Next, we examined the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. We observed constitutive hyperactivation of PI3K downstream targets in P1 B-cell progenitors relative to healthy control B-cell precursors (Figure 1G). Specifically, p4EBP1 and pAkt473 were significantly activated (12.46 ± 1.86 vs 4.00 ± 0.53 , $P = .0002$; 3.82 ± 1.78 vs 0.97 ± 0.09 , $P = .029$). However, no difference could be found in S6 phosphorylation (1.46 ± 0.14 vs 2.70 ± 0.51 , $P = .14$). 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 , $P = .006$). These results

Figure 1 (continued) the mutant alleles; half solid symbols, heterozygous persons; circles, female family members; square, male family members; double lines, consanguinity. (C) Expression of *FNIP1* messenger RNA in P1 (quantitative reverse-transcription polymerase chain reaction analysis). Data are expressed as mean $\pm$ standard deviation (2 independent experiments, each performed in triplicate). Statistical analysis was performed using 1-way analysis of variance. (D) *FNIP1* protein expression in T cells. (E) Bone marrow B-cell immunophenotyping in P1 compared with a healthy control and 1 Bruton tyrosine kinase (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). AU, arbitrary units. (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, ** $P < .01$ and *** $P < .001$. Data are presented as means $\pm$ standard deviation. AMP, adenosine 5'-monophosphate; ATP, adenosine triphosphate; HC, healthy control; WT, wild type.

Table 1. Immunological characteristics of patients with FNIP1 deficiency

	P1	Age-matched normal values	P2	Age-matched normal values	P3	Age-matched normal values
Age at the time of evaluation, y	3		9		25	
Coordinates (hg19) Chr5	Homozygous 131042150_G>A		Homozygous 130987494_C>T		Compound 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
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
Naive %	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
Naive %	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
Naive %	8.6	34.2-65.5	0.0	37.1-70.2	N/A	N/A
CD19 ⁺ 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

CADD, combined annotation dependent depletion; Chr, chromosome; Con A, concanavalin A; DC, dendritic cell; IL-2, interleukin 2; N/A, not available; Term. diff., terminally differentiated; WBC, white blood cell.

†Before G-CSF treatment.

#Before or without immunoglobulin replacement therapy.

Table 1. (continued)

	P1	Age-matched normal values	P2	Age-matched normal values	P3	Age-matched normal values
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
NK T (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 + IL-2	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	

CADD, combined annotation dependent depletion; Chr, chromosome; Con A, concanavalin A; DC, dendritic cell; IL-2, interleukin 2; N/A, not available; Term. diff., terminally differentiated; WBC, white blood cell.

†Before G-CSF treatment.

‡Before or without immunoglobulin replacement therapy.

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 1165 amino acids and shares 91% amino acid identity with human FNIP1.⁵ *Fnip1* plays a non-redundant 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 (ie, 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 2 patients, expanding the variant spectrum of this novel inborn error of immunity (supplemental Figure 10A). In fact, only a limited number of cases of chromosome 5 uniparental disomy have been reported.¹⁶ Our findings argue for the clinical use of exome sequencing with copy-number variant analysis in patients with complex phenotypes. Importantly, in 1 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 1 proband has had myopathy⁹ (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 1 individual had only decreased immunoglobulin M (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 (supplemental Figure 10B). Granulocytes are not affected in the *Fnip1*^{-/-} mouse,⁷ yet 4 patients have displayed severe or intermittent neutropenia, and 2 have monocytosis. This finding is reminiscent of the neutrophil survival defect seen in Bruton tyrosine kinase-associated agammaglobulinemia.¹⁸ As in other inborn error of immunity,^{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 4 out of 6 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.

Acknowledgments

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 (Houston, TX) 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 the Jeffrey Modell Foundation at Texas Children's Hospital (Houston, TX) for validation studies; Francesco Canonico from the Department of Neuroradiology, University of Milan-Bicocca, San Gerardo Hospital, ASST di Monza (Monza, Italy) for the brain computed tomography study; Francesca Pluchinotta and Antonella Camporeale from Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Donato (Milan, Italy) for the cardiac magnetic resonance imaging study; Giuseppe Limoli from Unità Strutturale Semplice Patologia Neonatale e Nido e Servizio di Cardiologia Pediatrica, ASST Lodi (Milan, Italy) for the cardiac ultrasound study; and GeneDx and GeneMatcher for facilitating collaboration.

This work was supported by the National Institutes of Health, National Human Genome Research Institute/National Heart, Lung, and Blood Institute (grant UM1 HG006542 to the Baylor-Hopkins Center for Mendelian Genomics), Fondecyt grant 11181222 (C.P.), Associazione Italiana Per La Ricerca Sul Cancro investigator grant 22082 (R.P.), "PG23/FROM 2017 Call for Independent Research" as part of the Rapid Assessment, Response, and Evaluation (RARE) project (to Papa Giovanni XXIII), Great Ormond Street Hospital National Institute for Health Research Biomedical Research Centre, and the Wellcome Trust (research grants 201250/Z/16/Z [E.R.] and 104807/Z14/Z [A.B.]).

Authorship

Contribution: F.S., C.P., L.D., C.B., M.I., Z.H.C.-A., and B.Y. designed and analyzed experiments; L.P.N., V.J.-C., A.B., G.F., M.Q., A.B.S., E.R., M.M., C.B., M.V., W.G.M.K., and M.M.v.O.-t.D. performed experiments; J.C.O., S.B., and 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., and G.G. were involved in study design, gave intellectual input, and critically revised the manuscript; D.M. conducted immunophenotyping on bone marrow and peripheral blood mononuclear cells; and F.S., C.P., I.K.C., J.V., F.H.R., and M.S. wrote the manuscript, which was approved by all authors.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: C.P., 0000-0001-6817-0573; J.V., 0000-0002-6291-935X; J.C.O., 0000-0001-9807-7273; F.H.R., 0000-0002-4833-3483; L.P.N., 0000-0002-6373-5376; C. Booth, 0000-0002-2626-5037; M.V., 0000-0002-7555-7706; A.B.S., 0000-0001-7538-4553; R.B., 0000-0001-7375-5410; C. Bugarin, 0000-0002-7401-7097; M.M.v.O.-t.D., 0000-0001-6772-7456; S.v.d.M., 0000-0001-8103-711X; R.P., 0000-0003-4198-9620; B.Y., 0000-0001-7278-5116; I.K.C., 0000-0001-5684-5457.

Correspondence: Francesco Saettini, Pediatric Hematology Department, Fondazione MBBM, University of Milano Bicocca, Via Cadore, 20900, Monza, Italy; e-mail: francescosaettini@yahoo.it.

Footnotes

Submitted 17 April 2020; accepted 22 August 2020; prepublished online on *Blood* First Edition 9 September 2020. DOI 10.1182/blood.202006441.

*I.K.C., L.D., and A.B. contributed equally to this study.

Data will be shared if requested to the corresponding author by e-mail.

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

REFERENCES

- 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.
- Baba M, Hong SB, 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 Natl Acad Sci USA*. 2006;103(42):15552-15557.
- Park H, Staehling K, Tsang M, et al. Disruption of Fnip1 reveals a metabolic checkpoint controlling B lymphocyte development. *Immunity*. 2012;36(5):769-781.
- Reyes NL, Banks GB, Tsang M, et al. Fnip1 regulates skeletal muscle fiber type specification, fatigue resistance, and susceptibility to muscular dystrophy. *Proc Natl Acad Sci USA*. 2015;112(2):424-429.
- Park H, Tsang M, Iritani BM, Bevan MJ. Metabolic regulator Fnip1 is crucial for iNKT lymphocyte development. *Proc Natl Acad Sci USA*. 2014;111(19):7066-7071.
- Siggs OM, Stockenhuber A, Deobagkar-Lele M, et al. Mutation of Fnip1 is associated with B-cell deficiency, cardiomyopathy, and elevated AMPK activity. *Proc Natl Acad Sci USA*. 2016;113(26):E3706-E3715.
- 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):1254-1261.
- 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;203(11):2899-2908.
- Niehues T, 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;50(7):1078-1080.
- Stray-Pedersen A, Sorte HS, Samarakoon P, et al. Primary immunodeficiency diseases: genomic approaches delineate heterogeneous Mendelian disorders [published correction appears in *J Allergy Clin Immunol*. 2018;141(2):832]. *J Allergy Clin Immunol*. 2017;139(1):232-245.
- 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.
- Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. 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.
- 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.
- Noordzij JG, de Bruin-Versteeg S, Comans-Bitter WM, 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.
- 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(4):791-793.
- 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.
- Centini R, Tsang M, Iwata T, et al. 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.
- 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):369-378.
- 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;189(4):e171-e174.
- Pasquet M, Bellanné-Chantelot C, Tavitan 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.