Journal of Clinical Immunology (2019) 39:353–357
https://doi.org/10.1007/s10875-019-00643-2

LETTER TO THE EDITOR

# *IKZF1* Loss-of-Function Variant Causes Autoimmunity and Severe Familial Antiphospholipid Syndrome

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Received: 8 March 2019 / Accepted: 6 May 2019 / Published online: 14 May 2019


To the Editor,

Ikaros is a transcription factor with key roles in lymphocyte development and homeostasis. It is encoded by the IKAROS family zinc finger protein 1 (*IKZF1*) gene, which contains highly conserved N-terminal and C-terminal zinc finger domains and is highly expressed in hematopoietic cells. The functions of Ikaros were first illustrated in different sets of *IKZF1*-deficient mice. In humans, *IKZF1* loss-of-function (LOF) somatic variants are associated with leukemogenesis in B cell acute lymphoblastic leukemia (B-ALL) [1]. More recently, different germline heterozygous variants, acting either by haploinsufficiency or by a dominant negative effect in *IKZF1*, were identified in autosomal dominant forms of common variable immunodeficiencies (CVID) or combined immunodeficiencies (CID) [2–4]. A series of families showed a progressive loss of B cells and hypogammaglobulinemia, but the absence of symptoms in several mutated patients suggests an incomplete penetrance. Additional germline mutations were described in patients with hypogammaglobulinemia associated with autoimmune manifestations, including vasculitis, juvenile-onset arthritis, or systemic lupus erythematosus (SLE) [5–7].

Here, we describe a two-generation non-consanguineous Caucasian family with three patients—the father (I.1), his son (II.1), and his daughter (II.2)—carrying a heterozygous *IKZF1* variant (c.500A>G; p.H167R) (Fig. 1a). The two children (II.1 and II.2) had antiphospholipid syndrome (APLS) in the context of a previous CVID and SLE diagnosis respectively, with autoimmune thrombosis and aberrant production of antiphospholipid antibodies (APLs) such as anticardiolipid, anti-β2gpI, or antiprothrombinase antibodies. Familial cases of APLS are very rare. One case of secondary APLS (in a juvenile-onset SLE patient) associated to another *IKZF1* deleterious variant has been already described [4]. We present the cases in the context of literature and discuss the potential implications of our findings.

## Case 1

The index patient (II.1) was diagnosed with juvenile-onset CVID at the age of 14 after a bacterial pneumonia and a septic shock. Polyvalent immunoglobulin replacement therapy was then initiated (IVIg). According to the pediatrician reports, his

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s10875-019-00643-2) contains supplementary material, which is available to authorized users.

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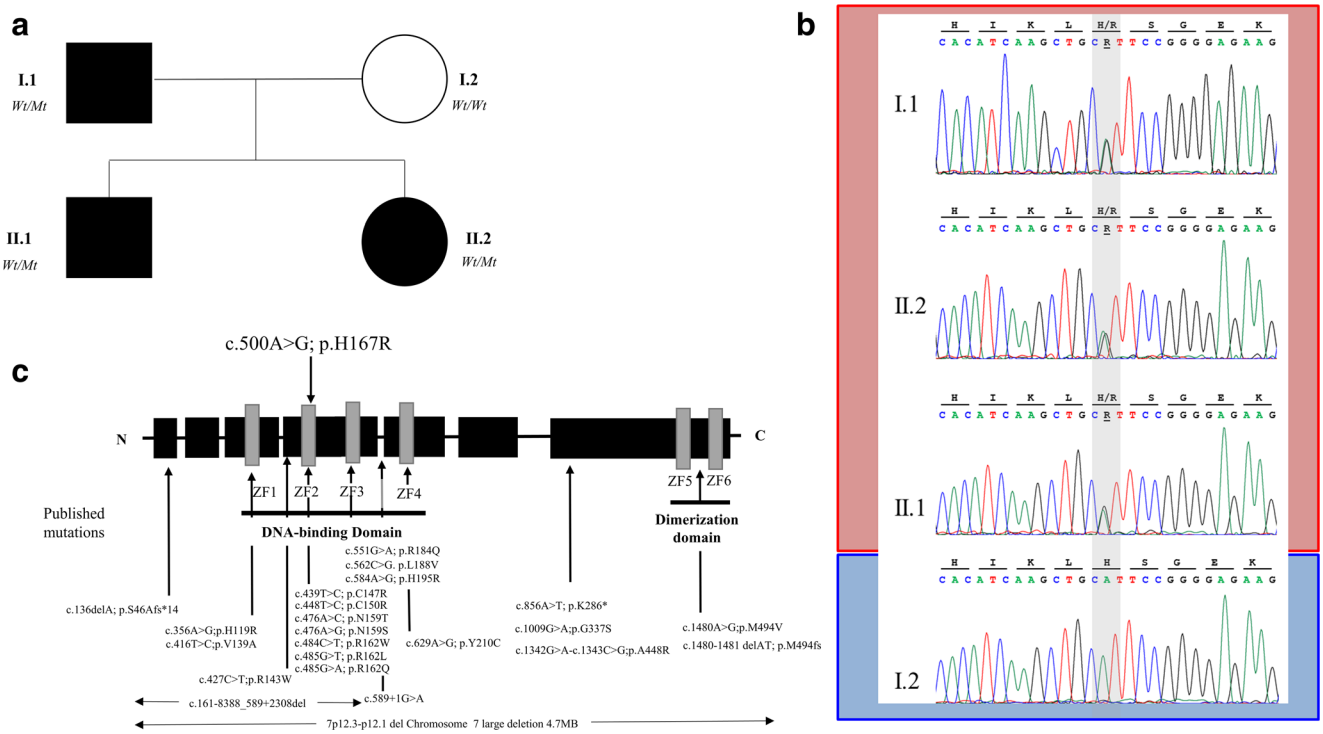


**Fig. 1** Genetic variants of the family with *IKZF1* mutation c.500A>G. Pedigree of the family (**a**), Sanger sequencing of *IKZF1* mutation (**b**), and localization of descripted mutations on *IKZF1* gene (**c**)

early medical history included a severe pneumonia that was rapidly followed by an adrenocortical hemorrhage, a reversible ischemic neurological deficit, a thrombosis of the left arm, and a transient renal failure with proteinuria. MRI revealed several vascular cortical defects of venous origin. The search for anticardiolipin antibodies and lupus anticoagulant (LA) was positive, allowing a diagnosis of APLS that was treated with anticoagulant and high doses of steroids. The diagnosis of catastrophic antiphospholipid syndrome was very likely considering that more than 3 organs were involved with renal failure and signs of disseminated intravascular coagulation. However, no biopsy was performed, in view of thrombocytopenia and the absolute need for anticoagulation [6]. He was first referred to the adult Clinical Immunology department (Strasbourg University Hospital) at the age of 19. At that time, he was free of infection and of any clinical manifestations, and his treatment consisted of prednisone, fluindione, and IVIg. ANA (antinuclear antibodies) were positive at 1/1280 without specific staining pattern. Antiphospholipid antibodies included high levels of IgM anticardiolipin and anti-β2gp1 antibodies, as well as LA (Table 1). Hypocomplementemia (low C3 and C4) was constant. The patient was then followed during several years under IVIg therapy and anticoagulant. Steroids were slightly decreased. He had at least 3 unexplained episodes of pneumonia that spontaneously regressed. He also presented several episodes of herpes infections and warts on both feet. He was then lost to follow-up. At the age of 24, the last laboratory examination showed normal Ig levels (under substitution) and still very high levels of IgM antiphospholipid antibodies with hypocomplementemia. He was again referred to our department at the age of 32 after the removal of a thrombosed implantable port, considering the occurrence of fever and a local hematoma. At that time, he was free of therapy except low-dose heparin in tandem with fluindione. He complained about abdominal and thoracic pain. Laboratory examination showed high levels of IgM APL antibodies, IgG APL antibodies, LA, hypocomplementemia, inflammatory syndrome, and high troponin level. Echocardiography and cardiac MRI confirmed cardiac microvascular disease with a favorable evolution under fluindione and aspirin. Further immunological analysis revealed subnormal levels of IgG, low lymphocyte counts with low CD4, and low B cell numbers. A more detailed immune profiling (Supplementary Fig. 1) revealed that memory $CD27^{+}$ B cells were particularly affected. $CD24^{+}CD38^{+}$ transitional B cells and $CD21^{low}CD38^{low}$ B cells (both considered to be possible reservoirs of autoreactive B cells) remained unchanged in percentages compared with healthy donors (HDs). CD4 lymphopenia was obvious with a subsequent decrease in all CD4 T cell subsets (Table 1 and Supplementary Fig. 1). However, memory T cells appeared more affected in percentages than in naïve and regulatory CD4 T cells (Tregs). Finally, as described for other CVID patients with auto-immune features, an important increase in the percentage of circulating T follicular helper type 1 (cTFh1) cells was observed [7].

**Table 1** Clinical and immunological features of affected individuals

| | I.1 | II.1 | II.2 | I.2 | Normal range |
|---|---|---|---|---|---|
| Age (years), sex (F/M) | 57, M | 32, M | 24, F | 57, F | |
| Age of onset (years) | Childhood | 14 | 11 | - | |
| Bacterial infections | + | + | + | - | |
| Other infections | - | + (HSV, HPV) | + (HSV, HPV) | - | |
| Autoimmune cytopenia | ITP | - | - | - | |
| SLE manifestations | - | - | Nephritis (class V), arthritis | - | |
| Other autoimmune diseases | - | - | - | - | |
| Thromboembolic events (A/V, arterial/venous) | - | + (A, V) | + (A) | - | |
| Auto-antibodies | + | + | + | - | |
| ANA (titer and pattern) | 1/1280 (nucleolar) | 1/1280 (nucleolar) | 1/1280 (speckled, nucleolar) | - | < 1/320 |
| Anti-dsDNA (titer, U/mL) | - | - | 313 | - | < 50 |
| Anticardiolipin (titer, U/L) | - | IgM (4880), IgG (62) | IgM (34), IgG (14) | - | 0–0.1 |
| Anti β2 GP-I (titer, U/L) | - | IgM (7191) | IgM (57) | - | 0–18 |
| Lupus anticoagulant | - | + | + | - | |
| Hypocomplementemia | + (C4) | + (C3, C4) | + (C3, C4) | - | |
| IgG (g/L) | 7.9 | 6.33 | 6.1 | NA | 7.2–14.7 |
| IgM (g/L) | ND | 2.75 | 1.14 | NA | 0.48–3.1 |
| IgA (g/L) | ND | < 0.22 | 0.49 | NA | 1.1–3.6 |
| Normal vaccinal response[a] | + | - | + | NA | |
| Lymphocytes (count/μL) | 1315 | 1038 | 1085 | NA | 1600–2400 |
| $CD3^+$ | 1218 | 849 | 936 | NA | 1100–1700 |
| $CD3^+CD4^+$ | 334 | 232 | 560 | NA | 700–1100 |
| $CD3^+CD8^+$ | 887 | 554 | 231 | NA | 500–900 |
| $CD4^+/CD8^+$ ratio | 0.4 | 0.4 | 2.4 | NA | 1.0–3.6 |
| NK $CD56^+$ cells | 56 | NA | 56 | NA | 200–400 |
| $CD19^+$ cells | 30 | 46 | 66 | NA | 200–400 |

[a] Normal vaccinal response was defined by adequate levels of antitetanus or antipneumococcal antibodies after vaccination considering laboratory reference values. *ANA*, antinuclear antibodies; *anti-dsDNA*, anti-double-stranded DNA; *F*, female; *HSV*, herpes simplex virus; *HPV*, human papillomavirus; *IgG/IgA/IgM*, immunoglobulin G/A/M; *ITP*, immune thrombocytopenic purpura; *M*, male

## Case 2

II.2 is the sister of II.1. She was diagnosed with juvenile-onset SLE and presented with arthritis and class V nephritis at the age of 11. Concomitantly, she was diagnosed with APLS with 2 episodes of chorea and triple positivity for APLs (LA, anticardiolipin, and anti-β2gI.1 antibodies, of both IgG and IgM subtypes). At that time, cerebral MRI showed several small bilateral ischemic lesions. Laboratory testing revealed slight hypogammaglobulinemia and elevated levels of IgM. ANAs were positive with anti-ds DNA antibodies and C3, C4, and hypocomplementemia. The treatment consisted of 1 mg per kg prednisone and efficient anticoagulation. Her condition improved with the exception of recurrent buccal herpes infections and warts on both hands as her brother. Four years later, she was referred many times with distal polyarthritis sensitive to steroid therapy. Hydroxychloroquine was not used due to two episodes of exudative retinal detachment. Prednisone was maintained at 5 to 10 mg per day to control the symptoms. At the age of 19, she was lost to follow-up. Her last treatment consisted of low-dose steroids, fluindione, and valaciclovir. She consulted in our department with her brother again at the age of 23. Laboratory values were stable with global lymphopenia (Table 1). She had no symptoms except for fatigue, subjective cognitive impairment, and trouble with attention. Physical examination was normal.

## Case 3

I.1 is the father of II.1 and II.2. Medical anamnesis revealed that he presented several infections in childhood including bronchopneumonia and sinusitis. He reported a thrombocytopenia episode, chronic fatigue symptoms, and fibromyalgia. Blood analysis showed low IgA and high titers of ANAs without anti-dsDNA antibodies. Anticardiolipin antibodies

were negative but LA was found. Lymphocyte subset analysis revealed a predominantly CD4 T cell lymphopenia (Table 1).

## Genetic Analysis and Discussion

Genetic variants were detected through whole exome sequencing (WES) of both patients II.1 and II.2, and their parents were filtered for rare coding variants predicted to be damaging (see supplemental material). The kindred's pedigree is shown in Fig. 1a. The father (I.1) and his children (II.1 and II.2) carried a c.500A>G substitution in *IKZF1* (NM_006060), resulting in a substitution of arginine for histidine at codon 167 (p.His167Arg). The segregation of the variant was confirmed by Sanger sequencing (Fig. 1b). No other deleterious mutations in genes known to be associated with monogenic forms of immunodeficiency or SLE were detected (Supplementary Table S1). Haploinsufficient variant c.500A>G (p.His167Arg) was previously reported as a LOF mutation in a family with three members affected by recurrent infections, but no autoimmunity. These mutated patients had a decrease in immunoglobulin levels, B cell lymphopenia, and an inverted CD4/CD8 T cell ratio, as in our patients. In this family, bone marrow examination indicated an incomplete blockade in B cell differentiation between the pre-B and the immature B cell stages [2].

To date, 77 patients with *IKZF1* LOF variants have been described in the literature (Supplementary Table S2). Most of them were affected by severe or recurrent bacterial infections [2, 3]. Reported heterozygous mutations in *IKZF1* (Fig. 1c) are associated with a decrease in *IKZF1* DNA binding leading to haploinsufficiency and a partial defect (CVID) [3–6, 8]. In some cases, heterozygous mutations of *IKZF1* lead to a dominant negative effect with a more severe phenotype (CID) [2]. In both cases, flow cytometry showed that the amounts of mutant proteins in T and B cells in cases of amino acid substitutions are comparable to those in cells from controls and that these mutant proteins are stable and able to enter into the nucleus. More recently, mutations have been reported in the C-terminal domain of IKAROS (dimerization domain) [7]. Twenty patients out of all patients described with *IKZF1* LOF variants (26%) suffered from autoimmune diseases. Autoimmune cytopenia (ITP and/or AIHA) was highly represented as in other PIDs [9]. All genotypes could be associated with autoimmune manifestations (Supplementary Table S3). In most cases, patients also developed infections and hypogammaglobulinemia, with progressive B cell lymphopenia and increased $CD8^+$ T cells with inverted CD4:CD8 ratio (mainly in case of protein-positive haploinsufficiency mutations) [2]. Among these *IKZF1* mutated patients with autoimmunity, Van Nieuvanhowe et al. reported a girl with the heterozygous *IKZF1* variant c.562C>G. This patient presented with juvenile-onset SLE and venous thrombosis due to APLS (LA, IgM, and IgG anticardiolipid antibodies but no anti-β2gpI antibodies). Systematic immunological analysis in the father also carrying the c.562C>G variant revealed the presence of LA and ANA [5].

To our knowledge, there is no unique explanation for autoimmunity in IKZF1 immunodeficiency. Both *Ikzf1*$^{-/-}$-null mice or *Ikzf1*$^{+/-}$-dominant-negative mouse models argue for a role of Ikaros in early B cell development stages in the bone marrow (resulting in B cell lymphopenia and hypogammaglobulinemia) but also in the control of central tolerance via mechanisms that remain to be elucidated [10–12]. Other evidences argue for a peripheral process of tolerance breakdown. Despite normal counts, B cells arbor phenotypical abnormalities including a decreased of switched memory B cells (Supplementary Fig. 1A), a hyperresponsive state (increase proliferation, increase of the expression of CD69 activation marker, Erk hyperphosphorylation, increase of CD19 and CD22) [5], and an increase of survival of autoreactive memory and long-lived B cells [5]. The role of TFh cells could be discussed, considering the relative increase of cTFh1 T cells (supplementary Fig. 1B) also found in other PIDs with autoimmunity [8, 13]. Furthermore, a direct relation between *IKZF1* variants and autoimmune SLE-*like* diseases is suggested both by clinical phenotype of IKZF1 immunodeficient patients and the association of common *IKZF1* polymorphisms in adult-onset SLE [14, 15].

Here, we describe 2 siblings with *IKZF1* mutation, presenting both a primary humoral and cellular immunodeficiency and APLS. One patient is affected with SLE and APLS (II.2), and the other one is considered to be primary severe APLS, likely CAPS (II.1) [16]. Hypothesis to explain the whole phenotype includes specific B and/or T cell tolerance impairment as well as a potential role of IKZF1 in other cells (such as myeloid cells or platelets) where the protein is also expressed. Based on these findings, we suggest that families with APLS and/or SLE with primary hypogammaglobulinemia could be explored for *IKZF1* mutations, particularly when they display viral or recurrent bacterial infections and an inverted CD4/CD8 ratio. Such a diagnosis would impact both the treatment and the follow-up considering the autosomal dominant inheritance and the risk for B-ALL.

**Acknowledgments** We thank C. Bole and S. Hanein (Institut Imagine) for exome sequencing. We thank JL Pasquali and P Kieffer for clinical data. We thank S. Bahram for critical reading. We thank S Jung for her helpful comments.

**Funding Information** A.S.K., A. G, and Y. D are supported by grants from the French Ministry of Health (PHRC IR 2011), from the Société Nationale Française de Médecine Interne (SNFMI), from the Hôpitaux Universitaires de Strasbourg (HUS), and from the EU-funded (ERDF) project INTERREG V "RARENET." R.C. is supported by the Agence Nationale de la Recherche (ANR) (ANR-11-LABX-0070_TRANSPLANTEX), MSD Avenir, and the INTERREG V European regional development fund (European Union) program (project 3.2 TRIDIAG).

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no competing interests.

## References


1. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, et al. BCR–ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature. 2008;453:110–4.
2. Kuehn HS, Boisson B, Cunningham-Rundles C, Reichenbach J, Stray-Pedersen A, Gelfand EW, et al. Loss of B cells in patients with heterozygous mutations in IKAROS. N Engl J Med. 2016;374:1032–43.
3. Boutboul D, Kuehn HS, Van de Wyngaert Z, Niemela JE, Callebaut I, Stoddard J, et al. Dominant-negative IKZF1 mutations cause a T, B, and myeloid cell combined immunodeficiency. J Clin Invest. 2018;128:3071–87.
4. Bogaert DJ, Kuehn HS, Bonroy C, Calvo KR, Dehoorne J, Vanlander AV, et al. A novel IKAROS haploinsufficiency kindred with unexpectedly late and variable B-cell maturation defects. J Allergy Clin Immunol. 2018;141:432–5 **e7**.
5. Van Nieuwenhove E, Garcia-Perez JE, Helsen C, Rodriguez PD, van Schouwenburg PA, Dooley J, et al. A kindred with mutant IKAROS and autoimmunity. J Allergy Clin Immunol. 2018;142: 699–702 **e12**.
6. Hoshino A, Okada S, Yoshida K, Nishida N, Okuno Y, Ueno H, et al. Abnormal hematopoiesis and autoimmunity in human subjects with germline IKZF1 mutations. J Allergy Clin Immunol. 2017;140:223–31.
7. Eskandarian Z, Fliegauf M, Bulashevska A, Proietti M, Hague R, Smulski CR, et al. Assessing the functional relevance of variants in the IKAROS family zinc finger protein 1 (IKZF1) in a cohort of patients with primary immunodeficiency. Front Immunol. 2019;10:568.
8. Turpin D, Furudoi A, Parrens M, Blanco P, Viallard J-F, Duluc D. Increase of follicular helper T cells skewed toward a Th1 profile in CVID patients with non-infectious clinical complications. Clin Immunol. 2018;197:130–8.
9. Fischer A, Provot J, Jais J-P, Alcais A, Mahlaoui N, Adoue D, et al. Autoimmune and inflammatory manifestations occur frequently in patients with primary immunodeficiencies. J Allergy Clin Immunol. 2017;140:1388–93 **e8**.
10. Kirstetter P, Thomas M, Dierich A, Kastner P, Chan S. Ikaros is critical for B cell differentiation and function. Eur J Immunol. 2002;32:720–30.
11. Macias-Garcia A, Heizmann B, Sellars M, Marchal P, Dali H, Pasquali J-L, et al. Ikaros is a negative regulator of B1 cell development and function. J Biol Chem. 2016;291:9073–86.
12. Wojcik H, Griffiths E, Staggs S, Hagman J, Winandy S. Expression of a non-DNA-binding Ikaros isoform exclusively in B cells leads to autoimmunity but not leukemogenesis. Eur J Immunol. 2007;37:1022–32.
13. Unger S, Seidl M, van Schouwenburg P, Rakhmanov M, Bulashevska A, Frede N, et al. The T H 1 phenotype of follicular helper T cells indicates an IFN-γ–associated immune dysregulation in patients with CD21low common variable immunodeficiency. J Allergy Clin Immunol. 2018;141:730–40.
14. He C-F, Liu Y-S, Cheng Y-L, Gao J-P, Pan T-M, Han J-W, et al. TNIP1, SLC15A4, ETS1, RasGRP3 and IKZF1 are associated with clinical features of systemic lupus erythematosus in a Chinese Han population. Lupus. 2010;19:1181–6.
15. Wang C, Ahlford A, Järvinen TM, Nordmark G, Eloranta M-L, Gunnarsson I, et al. Genes identified in Asian SLE GWASs are also associated with SLE in Caucasian populations. Eur J Hum Genet. 2013;21:994–9.
16. Legault K, Schunemann H, Hillis C, Yeung C, Akl EA, Carrier M, et al. McMaster RARE-Bestpractices clinical practice guideline on diagnosis and management of the catastrophic antiphospholipid syndrome. J Thromb Haemost JTH. 2018;16:1656–64.