

LETTER TO THE EDITOR

Activated PI3K δ syndrome type 2: Two patients, a novel mutation and review of the literature

To the Editor:

Gain-of-function (GOF) mutations in *PIK3CD* and *PIK3RI* encoding for the Class IA phosphatidylinositol 3-kinase (PI3K) subunits p110 δ and p85 α have recently been described in patients with childhood-onset combined immunodeficiency and were classified as activated PI3K δ syndrome (APDS) types 1 and 2, respectively (1–4). We report on the novel clinical, immunologic, and genetic findings in two patients with APDS type 2, thereby extending the phenotypic description of this probably under-diagnosed disorder. In addition, we reviewed the clinical features of so far published patients with APDS, thus pointing toward previously unnoticed clinical differences between APDS types 1 and 2.

Patient 1 (P1) is a currently five-year-old boy born at term to non-consanguineous Spanish parents (Fig. 1a). The family history was unremarkable for primary immunodeficiencies. At birth, intrauterine growth retardation (IUGR) with birth weight and height both below the 3rd percentile was observed. At 3 months of age, P1 suffered from severe RSV bronchiolitis requiring intensive care admission for transient noninvasive ventilation support. He recovered but subsequently presented with upper and lower respiratory tract infections during infancy. At 2 years of age, he started to develop chronic diarrhea, abdominal distention, and pain, primarily without pathogen isolation. At 3 years of age, he was transferred to his local hospital for diagnostic workup because of progressive failure to thrive, marked generalized lymphadenopathy, hepatosplenomegaly, and recurrent febrile episodes. A lymph node biopsy excluded an underlying malignancy and showed only unspecific reactive changes with predominant follicular hyperplasia. The initial basic immunologic screening identified a normal whole blood count, significantly reduced IgG and IgA, but marked elevated IgM levels for age. Because the expression of CD40L on stimulated T cells as well as CD40 was normal, the diagnoses of a hyper-IgM syndrome (HIGM) type I (CD40L deficiency) or III (CD40 deficiency) were considered unlikely. The initial management of P1 included sulfamethoxazole/trimethoprim prophylaxis plus intravenous immunoglobulin (IVIG) substitution resulting in normal for age IgG levels and transient normalization of the IgM level. However, respiratory tract infections persisted and he developed recurrent gastrointestinal tract infections (*Giardia lamblia* and *Campylobacter* spp.) despite progressively increasing IVIG doses and high IgG serum levels. Lymphadenopathy (cervical, axillary, and inguinal) as well as splenomegaly was repeatedly detected on clinical examination. A chest CT at the age of four demonstrated no abnormalities. CMV and EBV were neither detected in gut or lymph node biopsies nor in repeated blood PCRs. Upper and lower intestinal tract endoscopies revealed a severe erosive duodenitis, most likely secondary to *Giardia lamblia* infection for which he was successfully treated with

metronidazole; however, due to poor appetite, he failed to gain weight. Growth hormone (GH) substitution was initiated due to persisting short stature with borderline low GH and IGF-1 keeping in mind his clinical presentation of IUGR at birth. An extended immunologic screening at the age of four demonstrated normal total counts of T, B, and NK cells, but subset analysis identified reduced numbers of naïve CD4 T cells, an increase in senescent CD8 T cells, and reduced memory B cells. *In vitro*, T-cell proliferation after stimulation with the mitogen phytohemagglutinin (PHA) was moderately reduced (67% of an age-matched control). Details of the immunologic investigations are summarized in Table 1. In the context of this clinical and immunologic phenotype (i.e., reduced naïve T cells and impaired proliferation response), a combined immunodeficiency was assumed and the patient was included in a research program for undetermined benign lymphoproliferative disorders at the Center for Chronic Immunodeficiency, Freiburg (<https://www.uniklinik-freiburg.de/cci/studien/alps.html>). Next-generation targeted sequencing was performed and detected the heterozygous variation c.1425+2T>A in IVS11 of the *PIK3RI* gene (Fig. 1b). This variant was not listed in the reviewed databases (SNPbase, 1000 Genome Database, EVS Database, Kaviar Database, and ExAC Database). cDNA analysis with primers on exon 10 and exon 12 of *PIK3RI* revealed two equally intense bands of 341 and 215 bp (Fig. 1c). Sanger sequencing confirmed the wild-type sequence of the 341-bp band, while the 215-bp band had lost exon 11. The misspliced product predicts an in-frame deletion of amino acids 434–475. In the literature, patients with heterozygous variations in IVS10 of *PIK3RI* (according to our nomenclature: IVS11 c.1425+1G>T, IVS11 c.1425+1G>C, and IVS11 c.1425+1G>A) were previously described (2, 4–6). It was shown that these variations lead to splicing from exon 9 to exon 11 (according to our nomenclature: exon 10 to exon 12) (4).

Because of the reported benefit of mTOR inhibition in patients with APDS, rapamycin was introduced in P1 at the age of 5 (2 mg/m²/day, trough levels 8–12 ng/ml), resulting in a significant improvement of lymphoproliferation, weight gain, and growth, while awaiting the evaluation for possible hematopoietic stem cell transplantation in the future.

Patient 2 (P2) is a now five-year-old Spanish girl, born to non-consanguineous healthy parents (Fig. 1d), and presented aged 2 years with a suppurative cervical lymphadenopathy due to *Streptococcus pneumoniae* requiring intravenous antibiotics and repetitive surgical drainage. Similar to P1, immunology workup showed low IgG and IgA adjusted for age, elevated IgM, and reduced class-switched memory B cells. Absolute lymphocyte count and lymphocyte subsets as well as *in vitro* T-cell proliferation after PHA stimulation showed normal results. Senescent and naïve T cells were not

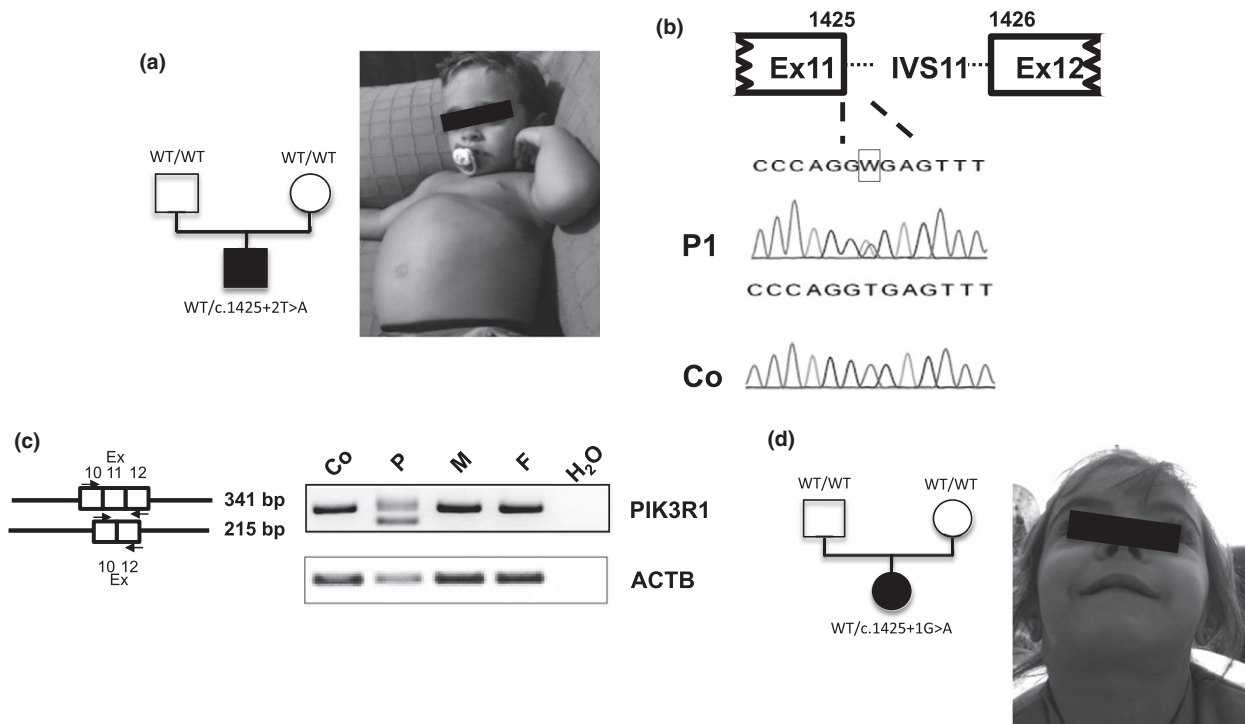


Figure 1 (a) Pedigree and clinical picture (severe hepatosplenomegaly) of P1. (b) Sequencing details of P1's *de novo* mutation (c.1425+2T>A) in IVS11 of *PIK3R1* (NM_181523.2). (c) Patient RT-PCR with primers on exons 10 and 12 of *PIK3R1* revealed two equally intense bands of 341 and 215 bp. Sanger sequencing confirmed the wild-type sequence of the 341-bp band, while the 215-bp band had lost exon 11. The misspliced product predicts an in-frame deletion of amino acids 434–475. (d) Pedigree and clinical picture (chronic cervical lymphadenopathy) of P2.

investigated in this patient. An initial presumed diagnosis of autosomal recessive HIGM type III was excluded. Due to the recently diagnosed patient 1 with *PIK3R1* mutation, targeted sequencing was performed and a previously reported c.1425+1G>A mutation in *PIK3R1* was identified. In addition to supportive management with IVIG substitution and sulfamethoxazole/trimethoprim prophylaxis, therapy with rapamycin (2 mg/m²/day) was initiated, leading to a marked decrease in lymphadenopathies but also onset of recurrent and severe aphthous stomatitis. Rapamycin was interrupted for four months and subsequently reintroduced with the aim to maintain serum trough levels between 8–12 ng/ml. Similar to P1, this patient is currently under evaluation for possible HSCT in the future.

Until recently, 49 APDS type 1 patients with in total four mutations in *PIK3CD* and 15 APDS type 2 patients with three mutations in *PIK3R1* (all affecting the same splice site) have been reported (1–12). In affected patients, augmented PI3K signaling has been shown to result in variable molecular consequences for the development and function of lymphocytes, including impaired immunoglobulin class-switch recombination and increased cellular senescence, leading to a progressive impairment of T- and B-cell memory generation and functions. To improve clinical awareness for these rare disorders, we have reviewed all hitherto published cases with APDS type 1 and type 2 for their clinical and immunologic symptoms; the key findings

are summarized in Fig. 2. As expected by the fact that the PI3K subunits p110δ and p85α are interacting subunits in the same signaling pathway, gain of function in these molecules results in an indistinguishable immunologic phenotype of, that is, hypogammaglobulinemia, progressive loss of B cells, reduced switched memory B cells, raised transitional B cells, progressive loss of naïve T cells, and increase in senescence T cells. All of these phenotypic alterations can be easily investigated by flow cytometry, and the addition of senescence markers (e.g., CD57/CD8 costaining) to routine lymphocyte subset panels may add important diagnostic value for the identification of patients with APDS among other individuals with benign lymphoproliferation. An example of our current flow protocol for senescent T cells (showing an increase in the percentage of senescent CD8 cells of P1) is provided in supplementary Fig. 1.

Our review of clinical features in APDS further confirmed many overlapping symptoms shared by type 1 and type 2 patients (Fig. 2). Clinical hallmarks similarly frequent in both cohorts are recurrent sinopulmonary bacterial infections, impaired virus control (i.e., of CMV and EBV), various signs of immune dysregulation (i.e., benign lymphoproliferation, autoimmune cytopenia, and susceptibility for lymphoid malignancies). Despite these multiple overlapping symptoms, we also identified previously unanticipated differences. Some of these (e.g., lower incidence of reported bronchiectasis in type 2 patients) might obviously be a bias of the retrospective nature of

Table 1 Summary of clinical and immunologic findings of P1 and P2

	P1		P2		Age-matched reference values	
	First visit (2 years)	At diagnosis (4.5 years)	First visit (2 years)	At diagnosis (5 years)		
Full blood count						
Hb (g/l)	95	93	90	113	118–157	
Neutrophils (cells/mm ³)	6.600	6.550	7.100	3.100	1.400–5500	
Lymphocytes (cells/mm ³)	3.900	3.470	5.900	3.400	3.000–13,500	
Monocytes (cells/mm ³)	1.800	1.160	1.200	400	100–1200	
Eosinophils (cells/mm ³)	10	1.480	430	830	50–700	
Platelets (cells/mm ³)	314.000	462.000	578.000	376.000	130.000–450.000	
PBMCs						
Lymphocyte (cells/mm ³)	3.421	3.604	5.101	3.981	1400–5500	
CD3+ T cells (%)	2.488 (76.1)	2.023 (61.4)	4.440	2.672	850–4.300 (52–92)	
CD4+ T cells (%)	975 (33.9)	952 (28.0)	1.846 (40.3)	1.347 (32.8)	500–2.700 (25–66)	
CD8+ T cells (%)	1.979 (68.7)	922 (27.1)	1.938 (42.3)	1.208 (29.4)	200–1.800 (9–49)	
CD4/CD8 ratio	0.49	1.03	0.95	1.12	1.1–2.5	
Double-negative T cells	0.80	–	ND	ND	<2.5	
CD3+/TCR α/β /CD4–/CD8– (%)						
CD3+/CD45RA+/CD45RO– (%)	24.0	–	ND	ND	52–92	
CD3+/CD45RA–/CD45RO+ (%)	22.1	–	ND	ND	0.26–9	
CD3+/CD45RA+/CD45RO+ (%)	52.9	–	ND	ND	0.28–2.0	
CD3 gamma/delta (%)	15.0	–	ND	ND	<10	
CD3+/CD57+/CD8+ (%)	ND	54.7	ND	ND	7–16	
CD19+ B cells (%)	229 (7)	180 (4.9)	327 (6.6)	349 (9.2)	0.9–38	
Memory CD19+/CD27+ (%)	58.9	17.4	48.2	62.5	8.1 – 33.3	
Naïve (CD19+/IgD+/CD27–)	27.0	59.0	38.9	31.4	59.7–88.4	
Non-class-switched memory cells (CD19+/IgD+/CD27+) (%)	8.5	3.3	21.3	7.5	3.1–18	
Class-switched memory cells		14.16	27.0	31.0	2.9–17.4	
CD19+/IgD–/CD27+ (%)						
Double-negative memory cells (CD19+/IgD–/CD27–) (%)	50.5	23.56	12.9	30	1.7–13.2	
Transitional B cells	14.1	60.64	21.6	9.9	2–70	
CD19+/CD24++/CD38++ (%)						
Plasmablasts	27.2	10.06	9.0	11.3	0.1–4	
CD19+/CD24–/CD38++ (%)						
CD38 low	43.1	9.7	21.2	37.0	2–16	
CD16+/CD3– NK cells (%)	1.3496 (15.2)	1.210 (32.8)	141 (2.9)	634 (16.7)	61–510 (2–25%)	
Albumin (mg/l)	4400	3500	4500	4000	3500–5300	
Immunoglobulins (mg/dl)						
IgG	75*	312	71*	714	2 years 424–1.051	4–5 years 463–1.236
IgA	<10	<10	<10	<10	14–123	25–154
IgM	780	155	769	258	48–168	43–196
Viruses (copies/ml)						
CMV	Negative	223	ND	857	Negative	
EBV	Negative	Negative	ND	Negative		
Mutation PIK3R1 IVS11	c.1425+2T>A		c.1425+1G>A			

*Prior IVIG substitution; ND, not done.

our review, a different focus of the clinical descriptions in publications, and due to the significant difference in patient numbers. Yet, the high incidence of microcephaly, poor growth/short stature (Fig. 2), so far primarily reported in patients with APDS type 2 (in some patients, including P1, even requiring GH substitution), certainly warrants clinical attention and may point toward a tissue-specific role of the p110 δ and p85 α factors. Interestingly in this regard, conditional deletion of

Pdk1, a kinase targeted by the p110 δ /p85 α complex, has been described to cause microcephaly in mice (13).

We further reviewed the treatment experience in APDS (Fig. 2). In the absence of long-term natural history data, the clinical management of APDS obviously remains an ongoing matter of debate. While the control of infections often requires immunoglobulin substitution, antimicrobial treatment, and prophylaxis, autoimmunity-associated organ damage may also

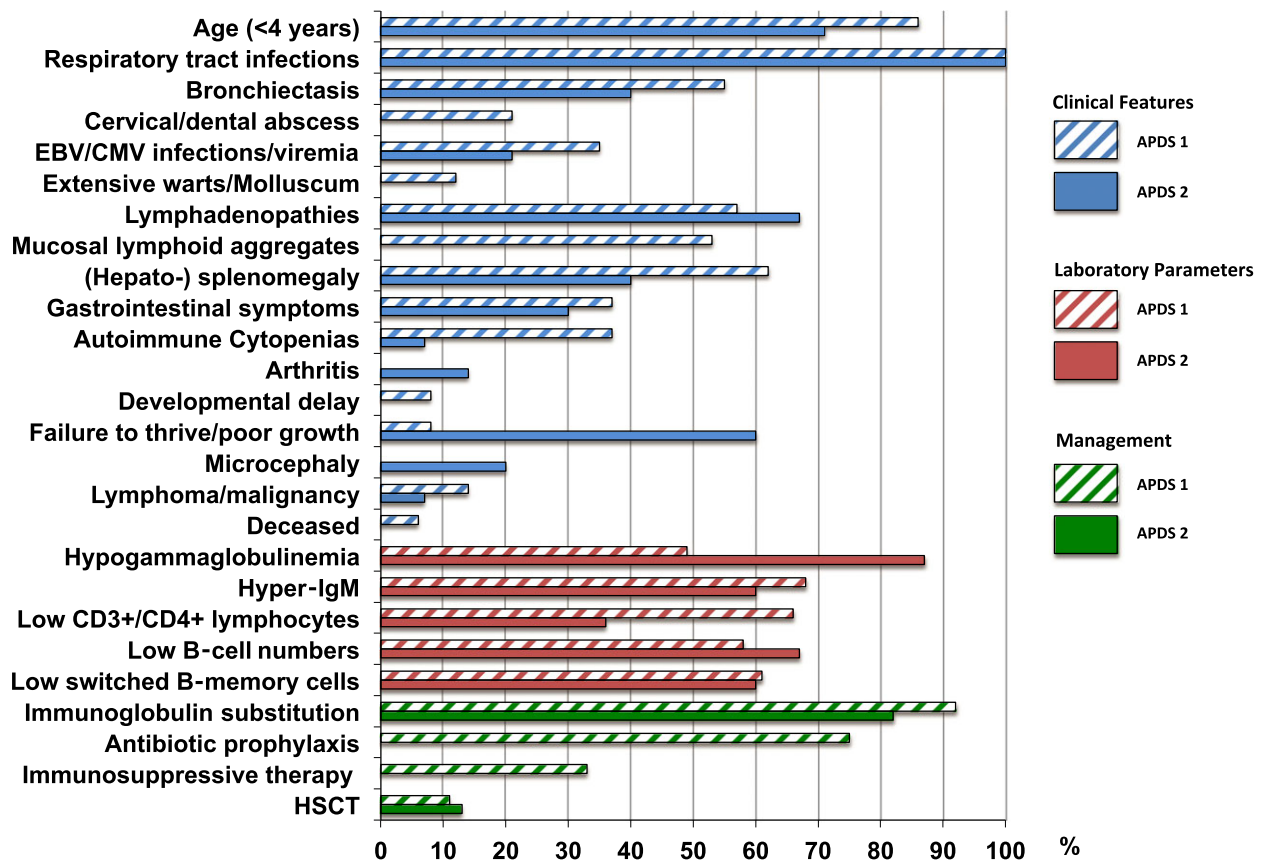


Figure 2 Overview of the reported clinical (blue) and laboratory phenotype (red) and therapeutic management (green) of previously reported patients (1–12) with APDS type 1 (light bars) and type 2 (solid bars). Information for each disease subtype is displayed as percent of reported patients (49 patients with APDS type 1 and 15 patients with APDS type 2).

demand continuing immunosuppression. Finding the right balance between immunosuppression and protection from infections remains an unsolved and often individual clinical challenge. In most previous reports, treatment details are sparse; particularly, the potential role of specific PI3K inhibitors in APDS patients still remains to be evaluated in prospective trials. At present, hematopoietic stem cell transplantation (HSCT) is the only reported curative treatment option (2, 3, 6, 12). To date, HSCT has been reported as being successfully performed in four patients (2, 3, 8, 12), while one patient who was splenectomized because of therapy refractory cytopenia died one year after transplantation from a septic shock (12).

Similar to other orphan diseases, future prospective and long-term follow-up data of patients with APDS are highly desirable to better understand the natural history and to optimize treatment concepts. A disease-specific registry, as currently set up by the European Society for Immunodeficiencies (ESID), is strongly warranted and will help the clinician in future decision making and better understanding of this disorder (<http://esid.org/Working-Parties/Registry/Studies/APDS-Registry>).

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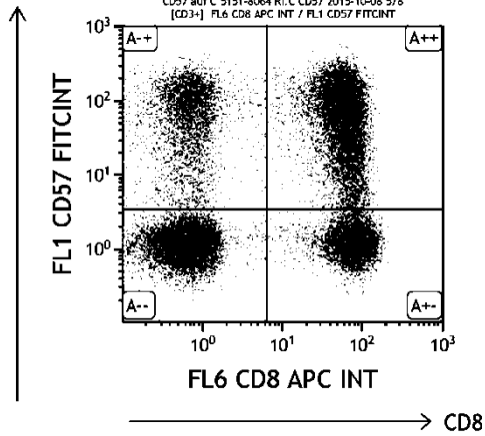
Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Whole blood was stained with anti-CD3 PerCp, anti-CD8 APC, and anti-CD57 FITC antibodies and analysed by flow cytometry after red blood cell lysis.

Supplementary Figure 1

CD57



CD57+/CD8+: 54,66%

Reference range (2-6years): 7,0-16%

Gate:
Lymphocytes/CD3