Activated Phosphoinositide 3-Kinase δ Syndrome: Update from the ESID Registry and comparison with other autoimmune-lymphoproliferative inborn errors of immunity

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Abstract

Background: Activated phosphoinositide-3-kinase (PI3K) δ Syndrome (APDS) is an inborn error of immunity (IEI) with infection susceptibility and immune dysregulation, clinically overlapping with other conditions. Management depends on disease evolution, but predictors of severe disease are lacking.

Objectives: Report the extended spectrum of disease manifestations in APDS1 versus APDS2, compare these to CTLA-4 deficiency, NFκB1 deficiency, and STAT3 gain-of-function (GOF) disease; identify predictors of severity in APDS.

Methods: Data collection with the European Society for Immunodeficiencies (ESID)-APDS registry. Comparison with published cohorts of the other IEIs.

Results: The analysis of 170 APDS patients outlines high penetrance and early-onset of APDS compared to the other IEIs. The large clinical heterogeneity even in individuals with the same *PIK3CD* variant E1021K illustrates how poorly the genotype predicts the disease phenotype and course. The high clinical overlap between APDS and the other investigated IEIs suggests relevant pathophysiological convergence of the affected pathways. Preferentially affected organ systems indicate specific pathophysiology: bronchiectasis is typical of APDS1; interstitial lung disease and enteropathy are more common in STAT3 GOF and CTLA-4 deficiency. Endocrinopathies are most frequent in STAT3 GOF, but growth impairment is also common particularly in APDS2. Early clinical presentation is a risk factor for severe disease in APDS.

Conclusion: APDS illustrates how a single genetic variant can result in a diverse autoimmune-lymphoproliferative phenotype. Overlap with other IEI is substantial. Some specific features distinguish APDS1 from APDS2. Early-onset is a risk factor for severe disease course calling for specific treatment studies in younger patients.

Clinical Implications

We report the largest APDS-cohort worldwide. APDS illustrates how a single genetic variant can cause a highly diverse autoimmune-lymphoproliferative phenotype overlapping with similar IEI. Early disease onset confers more severe disease.

Capsule summary

When comparing the phenotypic overlap of autoimmune-lymphoproliferative inborn errors of immunity (IEI) APDS demonstrates high penetrance, low genetic heterogeneity, early-onset as risk factor for severe disease and high phenotypic overlap with other IEIs.

Key words

APDS; PIK3CD; PIK3R1; PI3K; STAT3; CTLA-4; NFκB1; IEI; ESID; immunodeficiency.

Abbreviations

AD: autosomal dominant

AIHA: autoimmune haemolytic anemia

APDS: Activated phosphoinositide 3-kinase (PI3K) δ Syndrome

BCG: Bacillus Calmette-Guérin

CMV: cytomegalovirus

CTLA-4: cytotoxic T lymphocyte antigen 4

EBV: Epstein-Barr-Virus

ESID: European Society for Immunodeficiencies

GLILD: granulomatous-lymphocytic interstitial lung disease

GOF: gain of function

HPV: human papillomavirus

HSCT: hematopoietic stem cell transplantation

IEI: inborn error of immunity

315 NFκB1: nuclear factor of kappa light polypeptide gene enhancer in B cells

316 PASLI: p110-delta-activating mutation causing senescent T cells, lymphadenopathy, and
317 immunodeficiency

318 STAT3: signal transducer and activator of transcription 3

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Introduction

Activated phosphoinositide 3-kinase (PI3K) δ Syndrome (APDS), also called PASLI (p110-delta-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency), is an autosomal-dominant (AD) inborn error of immunity (IEI). Heterozygous gain-of-PI3Kδ-activity variants in *PIK3CD* or *PIK3R1* cause APDS 1 and 2 respectively (1–5), which show large phenotypic overlap. APDS is characterized by early-onset recurrent respiratory infections, chronic lymphoproliferation (benign and malignant) and other signs of immune dysregulation such as enteropathy and cytopenia (6–10). While previous cohort studies have illustrated a variety of clinical features of APDS, the identification and standardized documentation of additional patients allows extending the spectrum of disease manifestations that can be reliably associated with the two variants of the disease.

Interestingly, many clinical features of APDS are shared with other autoimmune-lymphoproliferative IEIs, including cytotoxic T lymphocyte antigen 4 (CTLA-4) deficiency (11–13), nuclear factor of kappa light polypeptide gene enhancer in B cells (NFκB1) deficiency (14,15) and signal transducer and activator of transcription (STAT3) gain-of-function (GOF) disease (16,17). All four IEI present an AD mode of inheritance, can cause increased infection susceptibility, early-onset benign lymphoproliferation, multisystem autoimmunity and an increased risk of lymphoma. Biomarkers facilitating diagnosis such as soluble FAS ligand and vitamin B12 for ALPS are lacking, rendering the differential diagnosis between these 4 IEI particularly challenging. However, a comparison of clinical manifestations between these conditions has not been performed. Delineation of entity-specific disease patterns can have diagnostic implications, while overlapping disease features may indicate pathophysiological convergence of affected signalling pathways, potentially offering opportunities for shared targeted interventions.

The clinical course of APDS is highly variable. While it can be life-threatening in childhood, stable disease into late adulthood has also been reported (6–8). This variability makes it difficult to advise patients about their individual prognosis and best treatment approach. The

most promising current therapeutic options include rapamycin, PI3Kδ inhibitors, and hematopoietic stem cell transplantation (HSCT) (8,18–22). Yet, the standard of care and use of these therapies in the long-term management of APDS patients remains to be defined. These interventions and their potential side effects must be balanced against the risks of the natural disease course. However, information on the natural history of APDS is still limited, and no clear risk factors for severe disease evolution have been identified.

In this study, we used an updated dataset of the European Society for Immunodeficiencies (ESID)-APDS registry of 170 patients with APDS and published datasets on other autoimmune-lymphoproliferative IEIs to address the following questions: (i) what are the clinical overlaps and characteristic differences between APDS, CTLA-4 deficiency, NFκB1 deficiency, and STAT3 GOF disease? (ii) are there differences in the spectrum of disease manifestations between APDS1 and APDS2? and (iii) can we identify early predictors of severe disease evolution in APDS patients?

Methods

The ESID-APDS Registry

The European Society for Immunodeficiencies (ESID) is a non-profit association whose aim is to improve knowledge in the field of IEIs. The APDS subregistry is the first level 3 dataset within the international internet-based ESID registry (https://esid.org/Working-Parties/Registry-Working-Party/ESID-Registry/The-3-levels-datasets-and-driving-questions). Documentation into the ESID-Registry is organized in three Levels. Level 1 is open to capture all IEI patients and includes a minimal dataset on initial manifestations, age at diagnosis, immunoglobulin replacement and HSCT with yearly follow-up on survival and changes in therapy (23). Level 2 allows to set up research projects that include some laboratory values and more details on treatments for a selected group of diseases. Level 3 allows to implement large datasets designed to address specific and extended clinical questions on a single IEI defined by a study protocol, including a statistical evaluation plan. All level 2 and 3 projects include level 1 data. Requirements for patients' registration are: positive vote from the local ethics committees; agreement between treating centre and ESID; signed ESID patient consent. Patient registration in the APDS subregistry also requires approval of evidence supporting the functional relevance of the mutation by one of the principal investigators. Patient data can be entered by authorized users via a standard web browser through encrypted communication (24). The first patient was registered in September 2015. The number of new patients documented per year is shown in Figure E1 A, the percentage of patients registered by the different countries in Figure E1 B.

Patients

46 centres collected data on 170 APDS patients (data closure for analysis: November 10th, 2022). 68 patients were already reported (8) (Table E1). The study was carried out in accordance with the recommendations of Section 15 of the Code of Conduct of the General Medical Council of Baden-Württemberg, Germany. The protocol was approved by the Ethics

committee of the University of Freiburg, Germany (IRB approval No. ESID registry: 493/14; IRB approval No. APDS registry: 458/15). All subjects or their parents/legal caregivers gave written informed consent in accordance with the Declaration of Helsinki.

To perform the comparison with other AD IEIs, the largest published cohort studies (13,15,17) were taken as reference and the frequency of reported clinical and immunological features were compared between all four IEI, since there are currently no level 3 ESID registry data on the other IEIs. A study proposal was written and was approved by the ESID registry steering committee, to collect level 1 data on the initial presentation of the analysed IEIs from the ESID Registry. Subsequently, complete data from patients whose documenting centres agreed to the protocol were included in the analysis.

Statistical Analysis

Data were exported and organized using Microsoft Excel (Microsoft, Redmond WA). Data visualisation and statistical analysis were performed using R version 4.1.0. Proportions between all IEI were compared using Pearson's chi-squared test. Analyses with a p value < 0.05 (*) were considered to be statistically significant. Only significant comparisons between all IEIs were shown in the figures. We performed a logistic regression to analyse the probability of severity in dependency of variables shown in Figure E4. For missing value imputation, we used the R package mice with predictive mean matching for numeric data and logistic regression imputation for binary data. To avoid overfitting, we performed bidirectional stepwise model selection by AIC. Weighted Cox Regression: Data are doubly truncated since the age at severity onset falls in the time interval between age at disease onset and age at study entry. We used inverse probability weighted Cox regression for doubly truncated data (25) to analyse the cumulative probability of severity in dependency of the binary variable age at onset under/over 1 year.

Results

APDS has low genetic heterogeneity, early onset and strong penetrance

Among the 170 APDS patients, 115 had heterozygous disease-causing variants in *PIK3CD* and 55 in *PIK3R1* (Table E1). Eight different disease-causing variants were found spanning p110δ with E1021K accounting for 90% (Figure 1A and 1B). All APDS2 patients carried deleterious splice site disease-causing variants resulting in “skipping” of exon 11 of p85α (Table E1). In contrast, 45 different *CTLA4* disease-causing variants were found among 133 patients (13), 56 disease-causing variants were identified in 157 NFκB1 deficient patients (15) and 72 different variants were reported in 191 STAT3 GOF patients (17). Thus, genetic heterogeneity of APDS appears to be lower compared to the other three IEIs. Median age at first clinical manifestation was 1 year in APDS patients, with no gender difference and no difference between APDS1 and 2. Age at onset was lower than that reported for CTLA-4 (median 11y) (13) and NFκB1 (median 12y) (15) deficiency, while patients with STAT3 GOF disease also presented early in life (median 2.3y) (17) (Figure 2A). The initial clinical manifestations experienced by APDS patients were most frequently infections (54%) and infections combined with immune dysregulation (29%), less frequently immune dysregulation without infections (8%) (Figure 2B). This was similar to NFκB1 deficiency (Figure 2B), while patients with STAT3 GOF and CTLA-4 deficiency more frequently first presented with immune dysregulation without infection (37% and 44%, respectively). Only 4 APDS patients were reported to be without clinical symptoms at registration (age at registration 1, 1, 3 and 44y), but two of them received immunoglobulin replacement for hypogammaglobulinemia. In the CTLA-4 and NFκB1 cohorts, 19.5% and 23% were reported to be clinically healthy, respectively. While unaffected STAT3 GOF carriers were not included in the Leiding cohort (17), a recent review (26) included 18% asymptomatic STAT3 GOF individuals. Hence, compared to these 3 other IEIs with overlapping phenotypes disease penetrance appears to be higher in APDS.

APDS has an earlier and more severe infection profile

Respiratory infections were frequent in all 4 IEIs with the highest occurrence in APDS (92%) (Figure 3A). Other common infections in APDS included invasive bacterial infections (53%) and infectious lymphadenitis (30%). Only one case of CMV-associated lymphadenitis was reported in the CTLA-4 cohort, and no cases were mentioned among the NFκB1 or STAT3 GOF patients. *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Staphylococcus aureus* were the most frequently reported respiratory pathogens in all diseases, while infections with *Pseudomonas aeruginosa* were reported more frequently in APDS (n=15/169) and STAT3 GOF (n=8/191). *Escherichia coli* and *Salmonella* were the most frequently isolated pathogens in bacterial intestinal infections. Chronic EBV (22%, age range 1-37y, median 5y) and chronic CMV (14%, age range 1-35y, median 8.5y) were present in APDS patients (Figure 3A). Similarly, in CTLA-4 deficient patients EBV and CMV led to clinically relevant infections in 18% and 10% respectively, while the reported incidence was below 5% in NFκB1 deficiency and STAT3 GOF. Acute viral infections were reported in 47% APDS patients. No cases of *Pneumocystis jirovecii* infection were reported in the APDS cohort and mycobacterial infections were rare (4 patients with Bacillus Calmette-Guérin (BCG) disease and 1 with pneumonia due to *Mycobacterium xenopi*). Parasitic infections were rare in all conditions; 2 cases of infection with *Cryptosporidium parvum*, 2 with *Giardia lamblia* and 2 with *Toxoplasma* were reported in the APDS cohort. Opportunistic infections were all prior to HSCT.

Bronchiectasis is more prominent than interstitial lung disease in APDS

143 APDS patients had chest imaging (CT-scan or MRI) performed: pathological findings were detected in 73%. Bronchiectasis was most frequent in APDS (50%, age range 1-43y; median 7y), but was also reported in the other IEIs (Figure 3B). Small airway disease was noted in 29% of APDS patients (age range 1-50y; median 8y). Interstitial lung disease (ILD) was only reported in 2% of APDS and in 7% of NFκB1 deficient patients. In contrast, CTLA-4 deficient patients were often (36%) reported to have granulomatous-lymphocytic interstitial lung

disease (GLILD) (Figure 3B). Similarly, ILD occurred in 43% of STAT3 GOF patients. Lung disease was severe enough to justify lung transplantation in 2 CTLA-4 patients and 2 STAT3 GOF patients. Interestingly, 30 APDS patients (18%) had asthma as concomitant diagnosis, compared to 6% in the CTLA-4 cohort and no reported cases in the other two cohorts. Lung function, assessed in 91 APDS patients, was abnormal in 47%.

APDS is characterized by chronic benign lymphoproliferation and early malignancy

Chronic benign lymphoproliferation, including both splenomegaly and persistent lymphadenopathy (defined as lymph nodes larger than 1 cm, affecting more than 1 site for longer than 1 month), was most frequent in APDS (86%), followed by CTLA-4 deficiency (73%) and STAT3 GOF disease (73%) with a lower incidence of 52% in NFκB1 deficiency (Figure 3C). Conversely, cytopenia was significantly less frequent in APDS (19%, most frequent: AIHA in 12 patients) than in CTLA-4 deficiency (62%), NFκB1 deficiency (43.9%), and STAT3 GOF disease (68%) (Figure 3C). Lymphoma was documented in 14% of APDS, 11% of NFκB1, 9% of CTLA-4 patients, but only 4% of STAT3 GOF patients (Figure 3D). Lymphomas in APDS included 7 Hodgkin lymphomas, 10 non-Hodgkin lymphomas, 1 intestinal large B cell lymphoma with plasmablastic differentiation, 1 follicular lymphoma, 1 large B-cell lymphoma, 1 mature T/NK lymphoma, 1 lymphoma without further histological information; 17/22 lymphoma cases were preceded by chronic benign lymphoproliferation. Of note, 10/20 lymphoma cases in APDS were EBV-associated. Moreover, of the 22 APDS patients with lymphoma, 4 suffered also from other malignancies (2 ovary neoplasms; 1 papillary renal cell carcinoma; 1 malignant neoplasm of the submandibular gland). Furthermore, one APDS patient had a B cell chronic lymphocytic leukaemia, one suffered from hepatocellular carcinoma, one had a breast ductal carcinoma *in situ*, one patient had a papillary thyroid carcinoma and one a rhabdomyosarcoma. The median age at diagnosis of any malignancy was much lower in APDS (19y) than in NFκB1 (46y) patients.

Autoimmune and inflammatory diseases are relevant in APDS, but less frequent than in the other diseases

Enteropathy, ranging from protracted diarrhoea to inflammatory bowel disease, was reported in 35% of APDS patients, less frequently than in the other IEIs (Figure 3E). Rare cases of eosinophilic oesophagitis and sclerosing cholangitis were also reported (27). Autoimmune hepatitis was particularly frequent in STAT3 GOF (Figure 3E). Non-infectious skin disease was reported in 25% of APDS patients and mainly included eczema and granulomas (Figure 3E). This was less prominent than in CTLA-4 deficiency (56%, mainly eczema) and STAT3 GOF disease (48% skin lesions including eczema, psoriasis and alopecia) but more frequent than in the NFκB1 cohort (15%), where patients suffered more frequently from skin infections. Endocrinopathies, including autoimmune thyroiditis and type 1 diabetes mellitus were reported in all four IEIs (Figure 3F) but were most frequent in STAT3 GOF disease. Renal disease affected 6-12% of APDS, CTLA-4 and STAT3 GOF patients, while it was not reported in NFκB1 deficiency. Moreover, 5 APDS patients were diagnosed with vasculitis and 2 different patients had systemic lupus erythematosus. One patient was diagnosed with chronic kidney disease, two received a kidney transplantation. Arthritis incidence was similar in all IEIs studied (Figure 3E). Less than 5% of APDS, STAT3 GOF and NFκB1 patients had inflammatory brain disease, while this was significantly more frequent in CTLA-4 patients (12%). In APDS non-inflammatory neurological manifestations including neurodevelopmental delay were observed in 16% of patients. Growth impairment was frequent in APDS (32%) and STAT3 GOF disease (57%), less frequent in CTLA-4 deficiency (14%), and not reported in NFκB1 deficiency (Figure 3F).

Increased immunoglobulin M and reduced naïve T cells are characteristic immunological abnormalities of APDS

Hypogammaglobulinemia was common in all four IEIs, but most frequent in NFκB1 deficiency. APDS is often characterized by elevated serum IgM (35%), while low IgM, a common feature

in the other 3 diseases, was rare in APDS (Figure 4A). While T-cell lymphopenia is common in all four IEIs, a low frequency of naïve CD4 T cells was most frequently reported in APDS. Reduced switched memory B cells and increased transitional B cells were reported but not particularly characteristic for APDS patients (Figure 4B).

Distinct features of APDS1 versus APDS2 indicate pathophysiological differences

Among initial presenting manifestations, syndromic features, mainly growth impairment and facial dysmorphism, were more frequent in APDS2 (Figure 5A; details are provided in Table E2). Infectious complications were equally distributed (Figure E2), but opportunistic infections were more frequent in APDS1. Significantly, bronchiectasis was more frequent in APDS1 (60%) than in APDS2 (26%) (Figure 5B). The prevalence of asthma was similar (18% vs. 16%). Splenomegaly and cytopenia were more frequent in APDS1 but lymphoma was more frequent in APDS2 (Figure 5C). Growth impairment was more frequent in APDS2, skin disease in APDS1 (Figure 5D). Among immunological abnormalities, low T-cell counts were more frequent in APDS1, while IgA reduction was more frequent in APDS2 (Figure 5E).

Age at first clinical presentation predicts disease severity in APDS

The majority of APDS patients received immunoglobulin replacement treatment (73%), many patients received immunomodulating therapies (Figure E3 A and B), ranging from rapamycin (37%) to PI3Kδ inhibitors (5%). 29/168 (17%) APDS patients underwent allogenic HSCT between the age of 5 and 51 years (median 13.5y). 14/170 (8%) APDS patients died at a median age of 18,5 years (5-44y). 5 deaths were lymphoma-related, 5 were HSCT-related, 1 related to both. Two patients died from severe respiratory infection, one from intracranial bleeding secondary to thrombocytopenia. To evaluate prognostic factors for a severe disease course in APDS, we defined severe disease as follows: (i) severe invasive infection *and* immune dysregulation (excluding chronic benign lymphoproliferation and cytopenia) *or* chronic lung disease, (ii) severe immune dysregulation, (iii) malignancy. If a patient had already developed a severe invasive infection or severe immune dysregulation or chronic lung disease

before age 13 years, the disease course was also considered severe. Criteria for severe disease were fulfilled by 93/169 patients (range 2-50y; median age at transition to severe disease 9.5y) (Figure 6A, Table E3). All deceased patients had severe disease with a median time between fulfilling these criteria and death of 6 years (range 1-21y). The risk for severe disease increased with patient age (Figure 6B) and with years since the first clinical disease manifestation (Figure 6C). The risk doubled in the age range 10-15 years compared to age range 0-10 years. Age at onset below 1 year significantly correlated with the probability of developing severe disease (Figure 6D). Other significant risk factors could not be identified through a multivariate logistic regression analysis (Figure E4).

Discussion

We report the evaluation of the so far largest APDS cohort of 170 patients with functionally validated, germline heterozygous variants in *PIK3CD* or *PIK3R1* documented through a standardised registry.

While highlighting the low genetic heterogeneity among APDS patients, we show that APDS1 patients, the majority of which carry the *PIK3CD* E1021K mutation, display high phenotypic diversity. This illustrates that identical variants in a disease causing-gene can lead to diverse clinical consequences. This emphasizes the significance of additional genetic, epigenetic and environmental factors in determining disease manifestations in autoimmune-lymphoproliferative diseases. This clinical variability is associated with a very high penetrance, as there was only one patient above the age of 5 years reported to be asymptomatic in the registry. However, systematic segregation studies would be needed in APDS as well as in the other IEI cohorts to better evaluate the true penetrance of these diseases and indirectly estimate the extent of underdiagnosed cases.

We structured the updated analysis of the APDS cohort in the context of a comparison with three other AD autoimmune-lymphoproliferative IEIs for which substantial cohorts have been published: CTLA-4 deficiency, NFκB1 deficiency, and STAT3 GOF disease. In general, there was a high clinical overlap between the investigated IEI, indicating relevant pathophysiological convergence of the different affected pathways. This convergence is supported by experimental observations: for example, a link between mTOR activation and disease pathophysiology is evident not only in APDS (4), but also in STAT3 GOF (28) and CTLA-4 deficiency (29). This justifies the frequent use of the mTOR inhibitor rapamycin in these three diseases, although variable treatment success indicates involvement of additional pathways. A potential link of mTOR activation to NFκB1 deficiency is less clear, mirrored by the reported use of rapamycin in only 2% of the patients in the largest published cohort (15).

Variability and overlap between the IEIs render it difficult to predict the diagnosis prior to genetic evaluation. However, some differences emerge from the comparative analysis. APDS

has the earliest onset, mainly with recurrent respiratory infections and this is in contrast to the frequent initial presentation with immune dysregulation typical of CTLA-4 deficiency and STAT3 GOF disease. Of note, the initial presentation with recurrent infections only rarely leads to the diagnosis of APDS, as recently highlighted by Ahmed et al. (30) who could diagnose only 1 APDS patient among 79 children admitted to the hospital for severe or recurrent respiratory infections. Infections are a crucial aspect in all 4 IEIs throughout the disease course, with highest frequencies observed in APDS and NFκB1 deficiency. These two conditions present mechanistically different but equally profound B-cell dysfunction (14,31–33). Regarding infections, it is important to note that regional exposure to different pathogens can influence the reported frequency of the infections. For example, a recent paper on a Chinese APDS cohort (34) reported a much higher incidence of primary mycobacterial infections than in this APDS series of patients. Chronic viral infections are confirmed to be relevant, especially in APDS and CTLA-4 insufficiency. On the other hand, our extended APDS registry cohort analysis reveals that opportunistic infections are rather rare in this disease.

Lung disease is a prominent feature in APDS and its early identification is crucial in the management of IEI patients. Of note, bronchiectasis and small airway disease were characteristic, while ILD was reported infrequently in APDS. It is important to note that small airway disease is likely underestimated in APDS, since specific expiratory imaging is needed for early detection (35). Importantly, asthma was recently pointed out as a relevant manifestation in an American APDS cohort (36) and had been already reported in some patients of small case series (37). The ESID-APDS registry does not specifically ask for asthma, but it was repeatedly documented as “further diagnosis”, thereby providing additional evidence to consider it an APDS-related manifestation.

Of the IEIs evaluated, APDS had the highest incidence of benign and malignant lymphoproliferation. This implies a diagnostic challenge of differentiating between benign and malignant lymphoproliferation (38). Imaging and FDG-PET do not provide a definitive

diagnosis, similar to other lymphoproliferative IEIs (39). For this reason, a thorough evaluation of the clinical course by experienced clinicians and an adequate histological analysis by pathologists trained in analysing lymphoid tissue of patients with IEIs is paramount to rule out lymphoma in these patients. The high incidence of non-lymphoid malignancies reported in our APDS cohort is noteworthy: while the increased risk of malignancy in IEI patients has long been known (40), increased awareness of APDS as cancer predisposition syndrome (41) calls for improved clinical care and research at the critical interface between immunology and oncology (42).

The analysis of the large APDS registry cohort also identifies arthritis, renal disease, neuroinflammatory disease or type 1 diabetes as rare but possible APDS-related complications. Overall, the differences between APDS and clinically overlapping IEI highlighted by our work are not sufficient to define a specific APDS-pattern or clinical diagnostic criteria for the disease. It is possible that including a higher resolution immunological analysis (such as high-dimensional multi-omics single cell data) may help identifying diagnostic biomarkers but at the moment, identification of a genetic variant in combination with its functional validation remains the only valid criteria.

Our analysis also highlights some new differences between the two forms of APDS corroborates others already noted through confirmation in a larger cohort and does not confirm others previously observed (6–8,36,43,44): thus, we report a significantly higher incidence of cytopenia and skin disease in APDS1 patients and a significantly higher incidence of reduced IgA in APDS2; we confirmed a higher incidence of bronchiectasis and reduced CD3 T cells in APDS1 and a higher incidence of lymphoma, growth retardation and syndromic features (detailed in this study) in APDS2. Regarding syndromic features, APDS2 can be differentiated from the SHORT (Short stature, hyperextensibility of joints and/or inguinal hernia, ocular depression, Rieger anomaly, and teething delay) syndrome, caused by mutations in the same gene (*PIK3R1*) but affecting another region (C-terminal Src homology 2 domain) resulting in a different effect (impairment of interaction with phosphorylated receptor tyrosine kinases)

(45). However, patients with overlapping clinical features have been reported (46–48). These clinical observations are relevant for the patient management and for research studies that further investigate pathophysiological differences between the catalytic and regulatory kinase components encoded by the mutated genes. Indeed, a recent work could identify relevant differences in B-cell abnormalities between APDS1 and APDS2 and highlight an increased perinatal mortality in APDS2 mice, but not in the APDS1 counterpart (49). Finally, a recently reported higher incidence of enteropathy in APDS1 patients and of elevated IgM in APDS2 patients (44) could not be confirmed.

It should be noted, that this registry analysis bears some relevant limitations: (i) The compared IEIs were not assessed using the same dataset, which may affect the reported frequency of some symptoms or diagnoses. (ii) Some manifestations are *per se* difficult to categorize, e.g. enteropathy can be difficult to distinguish from infectious enteritis. Internationally accepted standards of diagnosis and monitoring of these patients could help defining comparable data-sets and efforts are already taken in that direction (50). (iii) The registry- and the retrospective cohort study-structure are inevitably linked to the problem of missing data which leads to incomplete information and the eventual need of statistical corrections. In this study missing values were particularly relevant for laboratory parameters. Data completeness was only sufficient for some basic parameters, revealing that increased immunoglobulin M and reduced naïve T cells are characteristic, but not specific for APDS. It would be of interest to correlate more in-depth immunological parameters to identify possible disease-specific immune signatures and their role as prognostic factors.

One further aim of the current study was to identify predictors for severe disease in APDS, which could be useful for treatment and management choices. The number of variables evaluated as severe disease predictors was limited by the fact that many parameters were used in the definition of severe disease. Moreover, a registry-dependent bias in the identification and registration of younger patients with clinical symptoms of the disease must be taken into consideration, since the disease is not diagnosed through a screening but based

on clinical suspicion. The analysis revealed early disease onset as a prognostic factor, with the clinical implication that early-onset cases should be followed closely and evaluated early for treatments such as HSCT. It will be interesting to see in the future how targeted therapy with PI3Kδ inhibitors will impact on the long-term evolution of disease manifestations in APDS. Recent results of a phase 3 trial show promising efficacy, especially regarding the lymphoproliferative disease, with a very good safety profile (22). The poorer prognosis for patients with early disease onset identified in this study highlights the importance of clinical trials involving younger patients (such as the recently started NCT05438407).

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Figure Legends

Figure 1. Overview of the *PIK3CD* disease-causing variants in the registry. A, Localization of the variants in the *PIK3CD* gene. **B,** Frequency of the different variants. ABD = adaptor-binding domain. RBD = Ras binding domain.

Figure 2. Initial clinical presentation. A, Age at disease onset of APDS patients (median represented by the blue line, APDS patients represented by triangles). The median age at onset of NFκB1 deficiency (red), CTLA-4 deficiency (green) and STAT3 GOF (yellow) patients is superimposed as a dotted line. **B,** Initial clinical presentation of APDS patients (n = 170) compared to patients with NFκB1 deficiency (n = 83), CTLA-4 deficiency (n = 113) and STAT3 GOF (n = 41). Malignancy refers to both lymphoid and non-lymphoid malignancy. Data on all four IEIs were extracted from the ESID registry.

Figure 3. **Main clinical manifestations**. **A,** Main infectious complications of APDS patients (n = 170) compared to patients with NFκB1 deficiency (n = 121), CTLA-4 deficiency (n = 90) and STAT3 GOF (n = 191). **B,** Lung disease. **C,** Haematological complications. **D,** Malignancy. **E,** Other inflammatory manifestations. **F,** Endocrinological manifestations. * indicates p value < 0.05 in a t-test performed between every IEI. Data on NFκB1 insufficiency, CTLA-4 insufficiency and STAT3 GOF were extracted from published cohort papers.

Figure 4. Immunological abnormalities. A, Immunoglobulin abnormalities of APDS patients (IgG n = 145, IgA n = 137, IgM n = 137, IgE n = 56) compared to patients with NFκB1 insufficiency (n = n.a.), CTLA-4 insufficiency (n = 77) and STAT3 GOF (IgG n = 169, IgA n = 161, IgM n = 161, IgE n = 52). **B,** Cellular abnormalities of APDS patients (CD3 n = 152, CD4 n = 151, naïve CD4 n = 106, transitional B n = 46, switched memory B n = 83, NK cells n = 116) compared to patients with NFκB1 insufficiency (n = n.a.), CTLA-4 insufficiency (CD3 n = 44, CD4 n = 62, naïve CD4 n = 57, switched memory B n = 30, NK cells n = 61) and STAT3

GOF (CD3 n = 171, CD4 n = 169, naïve CD4 n = 31, switched memory B n = 31, NK cells n = 151). * indicates p value < 0,05 in a t-test performed between every IEI. N.a. = not available. Data on NFκB1 insufficiency, CTLA-4 insufficiency and STAT3 GOF were extracted from published cohort papers.

Figure 5. **APDS1 vs APDS2. A,** Initial presentation. Malignancy refers to both lymphoid and non-lymphoid malignancy. **B,** Lung disease. **C,** Haematological complications. **D,** Other inflammatory and endocrinological manifestations. **E,** Immunological abnormalities.

Figure 6. APDS disease evolution. A, Lexis diagram displaying all patients as lines from birth to time of last follow-up with the time of onset (blue dot), severity (red dot) and death (black dot). The line changes from gray to black at the time of entry into the registry (prospective observation). **B,** Cumulative probability of fulfilling criteria for a severe disease course with 95% confidence band; time scale is age in years. **C,** Cumulative probability of severe disease with 95% confidence band; time scale is years since onset. **D,** Weighted Cox regression to analyse the cumulative probability of severe disease depending on the variable age at onset </> 1 year.