**The broad clinical spectrum and unexpected features of Activated PI3-kinase Delta Syndrome; large patient cohort study**

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**Abstract**

**Background:** Activated PI3-Kinase Delta Syndrome (APDS) is a recently described combined immunodeficiency resulting from gain-of-function mutations in *PIK3CD,* the gene encoding the catalytic subunit of phosphoinositide 3-kinase δ (PI3Kδ).

**Objective:** To review the clinical, immunological, histopathological and radiological features of APDS in a large genetically-defined international cohort.

**Methods:** Clinical questionnaire, and review of medical notes, radiology histopathology and laboratory investigations of 53 APDS patients.

**Results:** Recurrent sino-pulmonary infections (96%) and non-neoplastic lymphoproliferation (75%) were common, often from childhood. Other significant complications included herpesvirus infections (49%), autoinflammatory disease (34%), and lymphoma (13%). Unexpectedly, neurodevelopmental delay occurred in 19% of the cohort, suggesting a role for PI3Kδ in the central nervous system (CNS); consistent with this PI3Kδ is broadly expressed in the developing murine CNS. Thoracic imaging revealed high rates of mosaic attenuation (90%) and bronchiectasis (60% in cohort); the incidence of bronchiectasis was greater than in common variable immunodeficiency (CVID). Elevated IgM (78%), IgG deficiency (43%) and CD4 lymphopenia (84%) were significant immunological features. No immunological marker reliably predicted clinical severity, which ranged from asymptomatic to death in early childhood. The majority of patients received immunoglobulin replacement and antibiotic prophylaxis, and five patients underwent haematopoietic stem cell transplant (HSCT). Five patients died from complications of APDS.

**Conclusion:** APDS is a combined immunodeficiency with multiple clinical manifestations, many with incomplete penetrance and others with variable expressivity. The severity of complications in some patients supports consideration of HSCT for severe childhood disease. Clinical trials of selective PI3Kδ inhibitors offer new prospects for APDS treatment.

**Clinical Implications:** The variable clinical phenotype with severe complications of bronchiectasis, bacterial and viral infections and lymphoma, suggests that patients who fit this clinical profile should be screened for the APDS-causing mutations.

**Capsule summary**

We describe complications, outcomes, management and laboratory and radiological features of APDS in the largest to date cohort of 53 patients, highlighting APDS as a clinically significant combined immune deficiency.

**Key words**

Activated PI3-kinase Delta Syndrome (APDS)

p110δ-activating mutation causing senescent T-cells, lymphadenopathy and immunodeficiency (PASLI)

Phosphoinositide 3-kinase δ (PI3Kδ)

*PIK3CD* gene

Bronchiectasis

Immunodeficiency

HSCT

PI3K Inhibitor

**Abbreviations**

AIHA Autoimmune haemolytic anaemia

Akt Ak strain transforming

APDS Activated PI3 kinase Delta Syndrome

BALF Broncho-alveolar lavage fluid

BCG Bacillus Calmette-Guerin

CF Cystic fibrosis

CMV Cytomegalovirus

CNS Central Nervous System

CT Computer Tomography

CVID Common Variable Immune Deficiency

EBV Epstein-Barr virus

HSCT Haematopoietic stem cell transplant

HSV Herpes simplex

mTOR Mammalian/mechanistic target of rapamycin

PI3K Phosphoinositide 3-kinase

PI3Kδ Phosphoinositide 3-kinase δ

PIP3 Phosphatidylinositol 3,4,5-trisphosphate

PTEN Phosphatase and tension homolog

PASLI p110δ-activating mutation causing senescent T-cells, lymphadenopathy and immunodeficiency

PCP Pneumocystis jirovecii pneumonia

PCR Polymerase chain reaction

PPV Pneumococcal polysaccharide vaccine

RTI Respiratory tract infection

RSV Respiratory syncytial virus

SAD Specific Antibody Deficiency

VZV Varicella zoster virus

**Introduction**

Activated PI3-Kinase Delta Syndrome (APDS) is an autosomal dominant primary immunodeficiency caused by gain-of-function (GOF) mutations in *PIK3CD*1,2, encoding the p110δ catalytic subunit of phosphoinositide 3-kinase δ (PI3Kδ). PI3Kδ, a Class 1 PI3K isoform, generating phosphatidylinositol 3,4,5-trisphosphate (PIP3), is a heterodimer comprised of p110δ and a p85-family regulatory subunit. PI3Kδ is expressed predominantly in leukocytes and plays an important role in their proliferation, survival and activation3-5*.*

Recently, we described 17 patients with a combined immunodeficiency disorder caused by the heterozygous *PIK3CD* GOF mutation E1021K1. Patients’ lymphocytes display increased basal and post-stimulation PIP3 and enhanced downstream Akt/mTOR signalling. This disorder was named **a**ctivated **P**I3-Kinase **d**elta **s**yndrome (APDS)1. Lucas *et al.* independently reported 14 patients with a similar disease caused by E1021K and two other activating mutations in *PIK3CD*, designating it PASLI (**p**110δ-**a**ctivating mutation causing **s**enescent T-cells, **l**ymphadenopathy and **i**mmunodeficiency)2*.* To date, 4 heterozygous GOF *PIK3CD* mutations (E1021K, N334K, E525K, C416R) have been described, with E1021K the commonest1,2,6-8. Patients in both cohorts suffered recurrent respiratory infections, bronchiectasis, herpesvirus infections, non-neoplastic lymphoproliferation and lymphoma. However, possibly due to different case-finding strategies, wereported bronchiectasis in 75% of our cohort and herpesvirus infections in 24%, whilst Lucas *et al.* described bronchiectasis in 33%, but all patients had herpesvirus viraemia. Recent reports have also underscored that APDS patients have a high incidence of lymphoma7,8 and possible autoimmune manifestations2,9*.*

In this study we describe the clinical, radiological, histopathological and immunological features of APDS in a genetically-confirmed cohort of 53 patients, the largest to date. We demonstrate a wide spectrum of clinical findings and complications, and unexpectedly note an increased frequency of neurodevelopmental manifestations. These findings will aid clinical decision-making in diagnosis and treatment of APDS, and facilitate patient counseling.

**Methods**

Informed consent was obtained from patients and/or parents. The study conformed to the Declaration of Helsinki and all local ethical requirements.

Mutations in *PIK3CD* were identified by Sanger sequencing1. Only patients heterozygous for an APDS-associated GOF *PIK3CD* mutation were included. Twenty-five patients from this cohort have been included in previous reports1,7and 28 are reported for the first time.

Information on demographics, presentation, complications, laboratory parameters, management and outcomes was compiled retrospectively by patient/parent interview and medical notes review. Pneumonia and bronchiectasis required radiological confirmation. Chest CT scans from 31 cases were independently reviewed by 2 thoracic radiologists (JB and NS) for air space opacity, atelectasis, nodules, bronchiectasis, mosaic attenuation and lymphadenopathy10,11*.* Available histopathology specimens (29 specimens from 11 patients) were reviewed by 2 haematopathologists (CMB and JRG). Patients’ most recent immunology results are described; post-Rituximab B-cell levels were excluded. All laboratory results were analyzed with reference to age-related normal ranges12-15. A poor pneumococcal polysaccharide vaccine (PPV) response was defined as <4-fold increase in anti-pneumococcal IgG titre, or <70% of serotypes >0.35ug/ml at 4-6 weeks post PPV vaccination.

Significant associations in clinical complications were determined by odds ratios with 95% confidence intervals (CI) and Fisher Exact tests using GraphPad Prism version 6. P values <0.05 were considered significant.

**Results**

**Patient characteristics**

Fifty-three APDS patients (34 male) from 30 unrelated families were included; 5 patients (4 male) were deceased. Living patients had a mean age of 17.2 (range <1-65) years. Forty-two patients were of European descent, 4 Afro-Caribbean, 3 Middle Eastern, 2 Indian, 1 Chinese and 1 Japanese. Fifty patients were heterozygous for E1021K and 3 related individuals were heterozygous for E525K.

**Presentation**

Recurrent respiratory infections occurred in 96% of patients with onset from <1-7 years of age. Lymphadenopathy and/or hepatosplenomegaly were common at presentation (42%). Five patients were identified in adulthood after their child was diagnosed with APDS; 2 had bronchiectasis and recurrent respiratory infections, 1 experienced recurrent respiratory infections in childhood and a persistent granulomatous local skin reaction to Bacillus Calmette-Guerin (BCG) vaccination, 1 was under investigation for chronic cervical lymphadenopathy but otherwise well, and 1 had no reported health issues. The 4 symptomatic adults had abnormal immunoglobulin profiles, including raised IgM and reduced IgG2, although none had a low total IgG.

**Infective complications**

*Bacterial infections*

Pneumonia (85%), bronchiectasis (60%) and upper respiratory tract infections were common, often with childhood onset (Table 1). Only 2 patients did not report recurrent respiratory infections. The commonest bacterial pathogens were *Streptococcus pneumoniae* and *Haemophilus influenzae,* with *Staphylococcus aureus*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and *Klebsiella* also observed. The mean age at diagnosis of bronchiectasis was 8.6 (range 1.3-36) years. Four patients developed permanent hearing loss from recurrent otitis media.

Non-respiratory bacterial infections included ocular infections (21%: conjunctivitis (n=8), dacryocystitis (n=3) and orbital cellulitis (n=2)) and abscesses (17%: *Staphylococcus aureus* skin abscesses (n=4), salivary gland abscesses (n=3), dental abscesses (n=3) and *Streptococcus pneumoniae* lymph node abscess (n=1)). No invasive bacterial infections were reported.

*Mycobacterial infections*

Two unrelated patients developed persistent granulomatous skin lesions at BCG vaccination injection sites (Figure 1); material from one lesion was culture-positive for BCG. No other mycobacterial infections were reported.

*Viral infections*

Persistent, severe or recurrent herpesvirus infections occurred in 49% of patients. Epstein-Barr virus (EBV) viremia was detected in 26%, with 6 patients (11%) developing disseminated infection, including one case of EBV encephalitis. EBV was detected in lymph node (n=3), tonsillar (n=1), palatal (n=1) and gastrointestinal (n=1) biopsies as well as cerebrospinal (n=1) and broncho-alveolar lavage fluid (BALF) (n=1). Two patients developed EBV-positive lymphoma. Eight patients suffered cytomegalovirus (CMV) viremia, 4 with systemic CMV infection successfully treated with ganciclovir. Four cases of EBV/CMV co-infection occurred. One patient (Figure 4.3) with diffuse lymphadenopathy and hepatosplenomegaly had EBV, CMV and human herpes virus 6 identified by PCR on lymph node biopsy. Two patients were hospitalized with severe primary varicella zoster virus (VZV) infection and two developed recurrent shingles. A non-genotyped sibling reportedly died of VZV pneumonitis aged 11 years. Recurrent herpes simplex virus (HSV) infections included oral ulceration (n=4), skin infections (n=2), and herpetic keratitis (n=1). HSV was identified in BALF of 2 symptomatic patients, one with severe pneumonitis.

Adenovirus infections were reported in 9 patients (17%), with positive isolates from blood, BALF and stool. Warts (n=4) and *Molluscum contangiosum* (n=4) were extensive in those affected.

*Parasitic and fungal infections*

*Cryptosporidium parvum* was isolated from a patient with bloody diarrhea aged 6-18 months, in whom cirrhosis was identified at age 8 years; the liver biopsy was negative for *Cryptosporidium*. A second patient developed *Cryptosporidium parvum-*positive diarrhea immediately post-HSCT. The only other parasitic infection identified was toxoplasmosis in a 9 month-old child. Oral mucocutaneous candidiasis requiring treatment was reported in 7 patients (13%) including candida tracheitis (n=1) and esophageal candidiasis (n=1). No cases of *Aspergillus* infection were identified.

**Non-infective immune complications**

*Non-neoplastic lymphoproliferation*

Chronic lymphadenopathy, splenomegaly and/or hepatomegaly were observed in 75% of patients (Table 1). Lymphadenopathy typically began in childhood, was persistent or recurrent, and often localized to sites of infection. There were 14 cases of cervical lymphadenopathy; 8 of 10 patients with persistent intrathoracic lymphadenopathy had bronchiectasis and recurrent consolidation. Seven patients had diffuse lymphadenopathy and EBV and/or CMV viremia was diagnosed in all 6 of these patients in whom viral PCR was performed. Lymphadenopathy was significantly associated with mucosal lymphoid hyperplasia (OR=16, CI=1.9-133.8, p=0.002), splenomegaly (OR=9.1, CI=2.5-33.2, p=0.0005) and herpesvirus infection (OR=6.9, CI=1.9-25.2, p=0.004).

Histologically (Figure 2), lymph nodes showed atypical follicular hyperplasia with absent or attenuated B-cell follicle mantle zones. Germinal centers were frequently disrupted and partially effaced by numerous T-cells, many of which were PD1 and/or CD57-positive, consistent with follicular helper T-cells. Parasinusoidal aggregates of monocytoid B-cells were a recurrent feature. IgG-positive plasma cells were reduced in number. One lymph node showed features analogous to post-transplant lymphoproliferative disorder, characterized by a polymorphic infiltrate of B-cells, T-cells, epithelioid macrophages and light chain-restricted plasma cells, monocytoid B-cell hyperplasia and equivocal immunoglobulin gene rearrangement assays. There was no progression to lymphoma on prolonged follow-up. Scattered EBV-positive and/or CMV-positive cells were present in several lymph nodes but florid infectious mononucleosis-like pathology was not encountered. Mucosal nodular lymphoid hyperplasia was visualized as cobblestone-like plaques or polyps in 17 (32%) of patients. In the gastrointestinal tract mucosal lymphoid hyperplasia was identified endoscopically from the epiglottis to rectum in 14 (26%) individuals, associated with diarrhea, bleeding and rectal prolapse. Five patients had respiratory mucosal nodular lymphoid hyperplasia identified bronchoscopically (Figure 2). Biopsies from mucosal lymphoid lesions showed follicular hyperplasia, often with features similar to those seen in lymph nodes, (Figure 2) and were occasionally PCR-positive for herpes viruses: EBV (n=1); HSV (n=1).

*Autoimmune and Inflammatory Disease*

Thirty-four percent of the cohort developed clinical features suggestive of autoimmune or inflammatory disease. Cytopenias included Coombs positive hemolytic anemia (n=7) and 2 cases of tri-lineage cytopenia, responsive to steroids or rituximab. Glomerulonephritis affected 3 children, necessitating renal transplantation in 2 cases. Renal biopsies showed proliferative, membrano-proliferative, and focal and segmental changes. Two patients developed exocrine pancreatic insufficiency. Autoantibody positive thyroid disease was diagnosed in 3 patients in adulthood. Two patients developed seronegative arthritis and one suffered recurrent pericarditis.

Three patients developed cirrhosis, of whom 1 also had sclerosing cholangitis in the setting of previous cryptosporidium diarrhea. Sclerosing cholangitis additionally affected a second, non-cirrhotic patient9*.* Thirteen patients (25%) had chronic diarrhea, nine of whom had gastrointestinal nodular mucosal lymphoid hyperplasia confirmed on endoscopy.

*Lymphoma and other malignancy*

Seven patients (13%) developed lymphoma, aged 18 months-27 years. There were 2 cases of diffuse large B-cell lymphoma, one EBV-positive (Figure S1) and one EBV-negative7. Single patients were reported as having nodular sclerosis classical Hodgkin lymphoma7, nodal marginal zone lymphoma1 and a lymphoplasmacytic lymphoma, the EBV status of which were unknown. An EBV-positive Hodgkin-type lymphoproliferative disorder was diagnosed in a child post-renal transplant. One child developed a primary cutaneous anaplastic large cell lymphoma (pcALCL) carrying a t(6;7)(p25;q23). This regressed from a 9 x 6 cm mass of tumour nodules to a 5x4 cm diameter flat erythematous plaque upon 6 weeks treatment with rapamycin (Figure S2). Three patients died of lymphoma-related complications including both patients with EBV-associated lymphoma. No other malignancies have been identified within our cohort to date.

*Neurological and other non-immune features*

Global developmental or isolated speech delay were diagnosed against standard criteria by specialist pediatric services in 10 patients (19%). Three further patients were treated for anxiety disorders, one was diagnosed with autism and 3 children were reviewed by psychological services for behavioral issues. Of note, PI3Kδ is strongly expressed in the mature and the developing murine central nervous system (CNS)16 (Figure 3).

Individual patients were born with macrocrania, unilateral hypoplastic kidney, and unilateral microphthalmia.

**Thoracic Radiology**

Air space opacity (Figure 4.1) was identified in 13 of 31 CT scans reviewed, and tree-in-bud opacities and/or bronchial wall thickening in 20. Mosaic attenuation was present in 28/31 patients, mild in 17, moderate in 7, and severe in 4 (Figure 4.2). Bronchiectasis was present in 21/31 scans with an average of 3 lobes affected, and associated with atelectasis or lobar collapse in 12 patients. Sixteen individuals had mediastinal lymphadenopathy, which was in a regional draining station to concurrent lobar consolidation in 4 instances. Follow-up imaging was available in 8 patients, at a mean interval of 2.2 years. Four of the patients with air space opacity and regional lymphadenopathy showed resolution of presumed pneumonic changes but persistent volume loss, atelectasis and development of bronchiectasis (Figure 4.1).

**Immunology laboratory results**

Lymphocyte immunophenotyping findings are summarized in Table 2. Typical findings were reduced CD4 T-cells, increased CD8 T-cells of an effector/effector memory phenotype and an expansion of transitional B-cells. A history of herpesvirus infection was not associated with a deficiency in NK-cells (p=0.48), helper T-cells (p=0.47), or cytotoxic-T-cells (p=0.35). Serial B-cell counts (n=19) suggest that patient B-cell levels fall more quickly over time than in age-matched controls (Figure 5).

Immunoglobulin levels (Table 3) were variable, with 43% of patients having reduced total IgG. 58% of patients with normal IgG had IgG2 subclass deficiency and 68% exhibited a poor response to polyvalent pneumococcal vaccine (PPV). Reduced IgA (50%) and elevated IgM levels (79%) were common. Two patients initially had marginally reduced IgM (aged 2 and 6 years), which over time became high (27g/l) or normal (0.63g/l) respectively. In 4 cases, high IgM normalized after commencement of immunoglobulin replacement. One patient developed a low IgG level following previous normal readings. Four patients with normal IgG and IgA responded poorly to PPV and were diagnosed with Specific Antibody Deficiency (SAD)17.

**Treatment**

*Anti-infection Prophylaxis*

Sixty-two% of the cohort currently, and an additional 9% previously, receive(d) antibiotic prophylaxis. Six patients (11%) are taking anti-viral and 3 (6%) anti-fungal prophylaxis.

*Immunoglobulin Replacement*

Long-term immunoglobulin replacement was administered to 87% of the cohort, with reported benefit (reduction of infection) in the majority. In 3 patients aged 14-23 years, immunoglobulin replacement was switched to antibiotic prophylaxis (patient preference). The 7 patients who did not receive immunoglobulin replacement therapy included the 5 individuals identified by genotyping relatives of APDS patients.

*Hematopoietic Stem Cell Transplant*

Five patients (9%), aged 5-14 years, have undergone HSCT with medium or reduced intensity conditioning with median follow up post-HSCT of 4.2 years (range 1-14 years). Three transplants (unrelated donors, one with 1A and 1B allelic mismatch) were successful, with minimal GVHD, restoration of normal growth and resolution of infection and non-neoplastic lymphoproliferation; chimerism in these individuals ranged from 35-100%. A fourth procedure was complicated by poor engraftment (25% donor chimerism) resulting in long-term immunoglobulin therapy post-transplant. A fifth patient, who underwent splenectomy prior to transplant, died of sepsis two years post-HSCT.

*Immunosuppression*

Thirty percentage of cohort underwent at least one course of immunosuppressive therapy for lymphoproliferative, autoimmune or inflammatory disease. Rituximab was of benefit in the management of AIHA (n=8) and non-neoplastic lymphoproliferation (n=5) though often complicated by sustained B-cell lymphopenia. Six patients were treated with rapamycin; 5 experienced benefit, with a decrease in non-neoplastic or neoplastic lymphoproliferation, but therapy was stopped in the fifth patient due to side effects.

**Fatal Outcomes**

Five APDS patients died, three (aged 1, 19, and 27 years) from lymphoma, 1 (aged 14 years) from sepsis post-splenectomy and HSCT and 1 (aged 39 years) from respiratory failure and chronic lung infection. Additionally, infection-related deaths in childhood and early adult life (≤30 years old) were reported for 5 non-genotyped relatives of APDS patients.

**Discussion**

We present an overview of the clinical course of APDS in the largest cohort to date with confirmed GOF *PIK3CD* mutations. The phenotype is highly variable (Figure 6), ranging from asymptomatic adults to profound immunodeficiency causing early death or necessitating HSCT in childhood; the clinical features overlap those of other PIDs such as CTLA4 and LRBA deficiency. Interestingly, 2 recent publications18,19 and Elkaim et al., (submitted) describe heterozygous mutations in the *PIK3R1* gene (encoding the PI3K regulatory subunit), leading to hyper-activation of PI3Kδ and a clinical syndrome (APDS2 or PASLI-R1) highly reminiscent of that described herein. Conversely, a recessive mutation in *PIK3R1* resulting in the loss of the p85α expression was reported in a patient with agammaglobulinemia and absent B cell lineage20. Together with the aberrant lymphocyte function in mice lacking PI3Kδ activity35, these findings indicate that balanced signaling in the PI3Kδ pathway is critical for normal immune function.

Recurrent respiratory infection is a near-universal manifestation of APDS. Bacterial isolates were typical for antibody deficiency, but the incidence of bronchiectasis was higher than in many CVID cohorts21,22 (Supplementary Table S1). Notably, 63% (20/32) of patients with bronchiectasis had normal total IgG levels, suggesting that patients with early-onset bronchiectasis and even minor immunoglobulin abnormalities should be screened for APDS mutations. Elevated IgM was seen in 82% of the cohort, reminiscent of class-switch recombination defect7,8. Thus we propose that patients presenting with reduced IgG and IgA with normal or elevated IgM17 particularly those with normal CD40L expression, should be screened for activating PI3Kδ mutations.

Almost half our cohort displayed difficulty in resolving herpesvirus infections, particularly EBV and CMV. There was no association between herpesvirus infections and decreased helper T-, cytotoxic T- or NK-cell counts, suggesting a functional defect underlies this susceptibility. Diffuse lymphadenopathy was associated with systemic herpes infections, with consistent features on lymph node histology. Other opportunistic infections were uncommon and patients did not develop PCP. *Cryptosporidium* was diagnosed in only 2 cases, one of whom developed cholangitis and liver disease, normally associated with MHC class II or IL21/IL21R deficiencies but also described in CD40L and CD40 deficiency23. Persistent granulomatous skin lesions after BCG vaccination occurred in 2 patients, but no other mycobacterial infections were reported. Although there was a moderate excess of skin infections and abscesses, there were no cases of invasive staphylococcal or *Aspergillus* infections to suggest major neutrophil dysfunction.

Although APDS can present as a CVID-like disease, it is also characterized by viral infections; lymphocyte immunophenotyping confirms APDS is a combined immunodeficiency. The typical T-cell profile was of reduced helper T-cells and recent thymic emigrants cells, while cytotoxic T-cells had a predominantly effector or activated phenotype. B-cell numbers were often normal in early life but fell with time. The reduction in B-cells, including class-switched memory B-cells and expansion of transitional B-cells suggests defects in B-cell maturation, or enhanced mature B-cell death35.

The development of focal bronchiectasis observed following consolidative changes strengthens the suspected causal link between infection and airway damage. Consistent with a role for infection in the florid non-neoplastic lymphoproliferation characteristic of APDS, lymphadenopathy was often associated with regional (mediastinal lymphadenopathy in bronchiectatic patients) or systemic infection (herpesviral infections), and tended to improve on infection resolution. Our review of chest CTs also revealed an unexpectedly high incidence (28/31) of mosaic attenuation, indicative of reduced perfusion of poorly ventilated lung regions. This may reflect inflammatory small airway disease or result from viral respiratory infections.

APDS patients had a high incidence (34%) and wide range of inflammatory/autoimmune manifestations. Enhanced PI3Kδ activity has been reported in autoimmune diseases such as SLE24, and PI3Kδ modulates regulatory T-cell function25. Our findings suggest a role for PI3Kδ in the genesis or perpetuation of autoimmunity, and potentially for PI3Kδ inhibition in treating such conditions. Activating somatic *PIK3CD* mutations have been associated with lymphoid malignancy26-28. We identified 7 lymphomas in this series of 53 patients, with a spectrum of pathological subtypes, but identified no solid malignancies, perhaps reflecting the young age of our cohort or the predominant expression of p110δ in leukocytes. Although PI3Kδ is described as ‘leukocyte-restricted’, expression is also found in cells of breast or melanocytic origin31, lung fibroblasts32 and TNFα-stimulated endothelial and synovial cells33. p110δ has recently been shown to regulate epithelial cell polarity34, of potential import for respiratory epithelial function. It is tempting to speculate that induction of p110δ expression by locally produced TNFα during inflammation might impair epithelial barrier functions and aggravate local inflammation. The lung phenotype might thus be the result of interplay between immune functions of p110δ and epithelial-intrinsic roles of p110δ.

Almost one fifth of our cohort experienced neurodevelopmental morbidity, from speech delay to global developmental delay. PI3Kδ is expressed broadly in the developing CNS, as well as in specific adult brain regions (including the hippocampus, cerebral cortex and thalamus) of reporter mice (Figure 3)16. PI3Kδ has been implicated in schizophrenia; pharmacological inhibition reversed prepulse inhibition deficits in a rat model of schizophrenia and blocked amphetamine-induced hyperlocomotion in a mouse model of psychosis-like behavior29. Interestingly, loss-off-function PTEN mutations (with consequent enhanced PI3K-dependent signaling) are associated macrocrania and autism-spectrum disorders30. One APDS patient had macrocrania, and in addition to the single patient with a formal diagnosis of autism in our cohort, prior to submission of this manuscript we were informed of an additional APDS patient with autism-spectrum disorder(personal communication; Professor P. Martin von Hagen, Erasmus MC, Netherlands). These findings suggest PI3Kδ may play an important but little-understood role in the CNS, and this aspect of APDS warrants further study.

HSCT has been seemingly curative in 3 APDS patients described herein and an additional 5 cases described by *Imai et al*36, supporting its use in carefully selected cases; however longer term follow-up to determine in particular the degree of donor chimerism need to achieve cure is needed. Lucas *et al*. reported a single patient in whom the mTOR inhibitor rapamycin improved circulating T cell profiles2. Four patients within our cohort experienced a decrease in non-neoplastic lymphoproliferation whilst taking rapamycin, and this drug also led to regression of a cutaneous T-cell lymphoma. Nevertheless, direct inhibition of the activated PI3Kδ may be a more attractive approach in APDS patients. Selective PI3Kδ inhibitors are currently in clinical trials for a range of cancers and inflammatory disorders, and one compound is already approved for treatment of B-cell malignancies25,26. Such disease-specific therapy could address both the infectious and non-infectious complications of APDS, but the reported side effect profile and significant immunoparesis in mice lacking PI3Kδ function32 emphasize the need for careful dosing to restore normal, rather than abolish PI3Kδ activity, particularly given that long-term treatment is contemplated.

**Conclusions**

APDS is a combined immune deficiency with variable phenotype, complicated by recurrent sino-pulmonary bacterial and herpesvirus infections, bronchiectasis, lymphoid hyperplasia, autoimmunity and, less frequently, neurodevelopmental delay and lymphoma. The rapidly increasing number of patients identified since the initial description of APDS in 2013 suggests this is a clinically significant cause of PID, which should be considered in patients presenting with atypical or inherited primary antibody deficiency, bronchiectasis, severe herpesvirus infections and lymphoma. The severity of complications and significant mortality rate support the consideration of HSCT in young patients as well as clinical trials of selective PI3Kδ inhibitors for this condition.

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**Figure Legends**

**Figure 1**. BCG-induced granulomatous inflammation in APDS. 1) Granulomatous skin lesion in a 4 year old at the site of BCG vaccination administered at 4 months of age. 2) Skin biopsy showing granulomatous inflammation (H&E stain, x100).

**Figure 2:** Lymphoid hyperplasia in APDS. 1-7) Lymph node histology showing atypical follicular hyperplasia (1, H&E, x20) with frequent disrupted follicles (2, H&E, x200, arrows) and monocytoid B-cells (arrowheads). Disrupted germinal centres are highlighted by staining for CD20 (3, x20; 4, x200) and Bcl6 (5, x200). Follicles were infiltrated by T-cells (6, CD3, x200) many of which expressed PD1 and/or CD57 (7, x200). IgM-positive plasma cells were present but IgG-positive plasma cells were reduced or absent (8, x200). Several lymph nodes contained CMV (immunohistochemistry) or EBV (EBER *in situ* hybridisation) (9, x200). 10-12) Mucosal lymphoid hyperplasia. Bronchoscopic image of trachea showing typical respiratory mucosal nodules (10). Pulmonary histology showing prominent peribronchiolar lymphoid hyperplasia (11, H&E, x40) with disrupted follicles as seen in lymph nodes (12, H&E, x100 /CD20, x200).

**Figure 3:** p110δ expression in the mouse brain. Brain sections of adult wildtype (- lacZ cassette) mice (left panel) and p110d kinase dead (+lacZ cassette) β-Gal reporter mice16 (right panel) stained with the neuronal stain cresyl violet (purple) and X-gal (blue) representing p110δ expression. Strong expression of p110δ was observed in areas of the hippocampus, cerebral cortex and thalamus.

**Figure 4.** Radiology of APDS. 1). Contrast-enhanced CT chest (2-year-old male), demonstrating marked localized right paratracheal lymphadenopathy (\*) on mediastinal windows (a), and right upper lobe consolidation and centrilobular nodules on lung windows (b). HRCT 2 years later (c) shows the development of severe right upper lobe bronchiectasis with persistent volume loss. 2). CT chest lung windows (a-c) in 7-year-old boy reveal mosaic attenuation (geographical areas of reduced attenuation and vascularity) indicative of small airways disease. Note mild right upper lobe bronchiectasis (a) and middle lobe/lingular atelectasis (black arrows in (b). 3). Contrast-enhanced CT chest (a), abdomen (b) and pelvis (c) in an 8-year-old boy showing bilateral axillary, right paratracheal, para-aortic (black arrow), mesenteric (\*) and bilateral inguinal lymphadenopathy (white arrows) and splenomegaly.

**Figure 5:** Age-related changes in B-cell counts in APDS. Age-related median B-cell count (white line), B-cell count 5-95th percentile normal range (checked area) and less than 5th percentile normal B-cell count (spotted area) plotted.12

**Figure 6:** Variation in clinical phenotype of APDS. Each column represents a patient with APDS. Each row represents a frequent or serious complication of APDS. White boxes and grey boxes depict the absence or presence of a complication respectively.