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The First Iranian Cohort of Pediatric Patients with Activated Phosphoinositide 3-Kinase-δ (PI3Kδ) Syndrome (APDS)

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ABSTRACT

Background: Activated phosphoinositide 3-kinase δ syndrome (APDS) is a recently defined combined primary immunodeficiency disease (PID) characterized by recurrent respiratory tract infections, lymphoproliferation, autoimmunity and lymphoma. Gain-of-function mutations in *PIK3CD* and loss-of-function of *PIK3R1* genes lead to APDS1 and APDS2, respectively.

Methods: Demographic, clinical, immunological and genetic data were collected from medical records of 15 pediatric patients, who were genetically identified using the whole-exome sequencing method.

Results: Fifteen patients (6 APDS1 and 9 APDS2) were enrolled in this study. Recurrent respiratory tract infections followed by lymphoproliferation and autoimmunity were the most common manifestations (86.7%, 53.3% and 26.7%, respectively). Five patients (33.3%) had a Hyper-IgM-syndrome-like immunoglobulin profile. In the APDS1 group, splice site and missense mutations were found in half of the patients and the C-lobe domain of *PIK3CD* was the most affected region (50%). In the APDS2 group, splice site mutation was the most

KEYWORDS
Primary immunodeficiency disease; activated phosphoinositide 3-kinase δ syndrome (APDS); phosphoinositide 3-kinase δ; *PIK3CD*; *PIK3R1*

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frequent mutation (77.8%) and the inter-SH2 domain was the most affected region of *PIK3R1* (66.7%). Mortality rate was significantly higher in APDS2 group ($P = .02$) mainly due to chronic lung infections. **Conclusion**: Respiratory tract infections and humoral immunodeficiency are commonly the most important complication in pediatric APDS patients, and they can be fatal by ultimately causing catastrophic damage to the structure of lungs. Hence, physicians should be aware of its significance and further work-up of patients with recurrent respiratory tract infections especially in patients with lymphoproliferation. Moreover, delineation of genotype-phenotype associations with disease severity could be helpful in the timely application of appropriate management and patients' survival.

Introduction

Activated phosphoinositide 3-kinase-δ syndrome (APDS) is a newly described primary immunodeficiency disease (PID) (Lucas, 2014a). There are two subtypes of APDS including APDS1 and APDS2. APDS1 is caused by monoallelic activating mutations in the *PIK3CD* gene while APDS2 is caused by loss-of-function mutations in the *PIK3R1* gene. *PIK3CD* and *PIK3R1* encode for p110δ catalytic and p85α regulatory subunits of class IA phosphoinositide 3-kinase δ (PI3Kδ), respectively (Deau et al. 2014, Lucas, 2014, Lucas, 2014). PI3Ks are a family member of the intracellular downstream signaling transducer enzymes (Fruman and Bismuth 2009). PI3Kδ is predominantly expressed in leukocytes (Okkenhaug and Vanhaesebroeck 2003). Upon activation of PI3K downstream of cytokine receptors, B cell receptors (BCR), T cell receptors (TCRs) and Toll-like receptors (TLRs), phosphatidylinositol 4,5-biphosphate (PIP2) is phosphorylated and converted to phosphatidylinositol 3,4,5-triphosphate (PIP3). Then, PIP3 activates phosphoinositide-dependent protein kinase-1 (PDK1) and AKT, thereby enhance the mechanistic target of rapamycin (mTOR) signaling pathway and inhibition of the Forkhead box family (FOXO) of transcription factors (Sauer et al. 2009). Hence, PIK3δ plays an important role in lymphocyte functions including cell growth, proliferation, differentiation, motility, survival and intracellular trafficking (Koyasu 2003; Okkenhaug and Vanhaesebroeck 2003). The underlying APDS-related mutations lead to hyper-activation of the PI3K signaling cascade and consequently clinical and immunological manifestations of the condition (Lucas et al. 2016).

APDS patients present a wide spectrum of clinical phenotypes ranging from mild to fatal. Symptoms and signs include recurrent respiratory tract infections, bronchiectasis, chronic non-resolving infections with Herpesviridae family viruses, non-neoplastic lymphoproliferation, autoimmunity, increased risk of lymphoma, and neurodevelopmental abnormalities (Elkaim et al. 2016, Lucas, 2014; Singh et al. 2020). Immunological phenotypes of these patients is variable, but typically consist of low/normal serum level of immunoglobulin A (IgA) and IgG, normal or increased IgM (Fekrvand et al. 2020), decreased total and naïve $CD19^+$ B cells with increased transitional B cells, reduced $CD4^+$ helper T cells, normal to increased $CD8^+$ cytotoxic T cells with an increased senescent proportion, and normal to decreased natural killer cells (Lucas, 2014, Singh et al. 2020). Management of the affected patients varies based on the severity and extent of clinical features. Therapeutic options

include antibiotic prophylaxis, immunoglobulin replacement therapy, immunomodulators, PI3Kδ inhibitors and hematopoietic stem cell transplantation (HSCT) (Azizi et al. 2016; Coulter et al. 2017; Elkaim et al. 2016).

There we report the first cohort study of Iranian pediatric patients with APDS with demographic, clinical, immunological and genetic characteristics of these patients in order to further elucidate the complex aspects of this newly identified disorder.

Materials and methods

Study population

A total number of 15 patients with APDS were included in the study. Patients were referred to Children's Medical Center (Pediatrics Center of Excellence affiliated to Tehran University of Medical Sciences, Tehran, Iran) by the Iranian Primary Immunodeficiencies Network (IPIN). All patients were under clinical follow up based on the national consensus management for PID (Abolhassani 2019b). The diagnosis of APDS was confirmed based on molecular diagnosis and presence of mutations in *PIK3R1* or *PIK3CD* genes confirmed by the whole-exome sequencing (WES) method as explained in detail previously (Abolhassani 2019a, 2020). Secondary causes of immunodeficiency and all known genes associated with primary immunodeficiency (Tangye et al. 2020) were excluded in all patients with or without parental consanguinity. The Ethics Committee of the Tehran University of Medical Sciences approved this study and written informed consent was obtained from all patients and/or their parents.

Data collection

A two-page questionnaire was designed to retrospectively obtain all demographic, clinical, laboratory and genetic information from the patients' medical records. Demographic data (current age, vital status, age at onset of symptoms, age at diagnosis, delay in diagnosis and course of the disease), clinical manifestations (first presentation, respiratory complications including pneumonia, sinusitis and otitis media, and non-respiratory complications including lymphoproliferation, autoinflammatory disorders, malignancy, and involvement of gastrointestinal, ophthalmologic, neurologic, rheumatoid and cutaneous systems), as well as immunologic and genetic data were included. We determined medical severity in each patient based on the severity of infections, the presence of malignancy and the vital status of the patients. All patients received regular intravenous Ig (IVIg) replacement therapy with doses of 400–600 mg/kg/month, as well as prophylactic antibiotic therapy according to the type of disease in those with refractory diseases (>3 breakthrough infections or extremely severe infection on IVIg, bronchiectasis, chronic sinusitis and recurrent acute otitis media).

Statistical analysis

Statistical analysis was accomplished using SPSS software (SPSS Inc., version 24, Chicago, IL, USA). Kolmogorov–Smirnov and Shapiro–Wilk tests were used to test for the data normality. Central and descriptive statistics were reported for quantitative

data. For variables with skewed distribution, median and interquartile ranges (IQR) were reported as the index of data exact tests. Different survival estimates were compared using Kaplan–Meier curves and log-rank tests. *P value*<.05 was considered statistically significant.

Results

Demographic features

A total of 15 patients (male to female ratio, 1:2) with median (IQR) age of 22 (9.7–32.0) years at the time of the study were enrolled. Six patients were diagnosed to have APDS1 and the remaining 9 patients APDS2. The median (IQR) age at the onset of disease was 3 (2–4) years across the study population, with respiratory tract infections as the most common first clinical presentation in both APDS1 and APDS2 groups followed by lymphadenopathy, diarrhea, cutaneous and nail lesions (Figure 1). The patients were followed up for a median (IQR) of 84 (48.0–204.0) months. At the time of the study, eight patients (53.3%) were alive, while six patients (40%) were dead and the vital status of one patient (6.7%) was unknown due to loss of follow-up. Of note, 5 (83.3%) of the deceased patients were diagnosed with APDS2. The mean for survival time in APDS2 patients was shorter compared to APDS1 group, although this difference was not significant [mean (95% confidence interval): 167.2 (112.9–221.5) months vs. 204 (102.2–305.8) months, respectively, P = .522] (Figure S1). Five patients had a positive family history of recurrent infection, all of whom were APDS1 and this difference was statistically significant compared to the APDS2 group (83.3 versus 0%, P = .002). Detailed demographic data of the studied patients, as well as their comparison between APDS1 and APDS2 groups, are shown in Table 1. Except for positive family history, there was no significant difference in demographics between APDS1 and APDS2 groups.

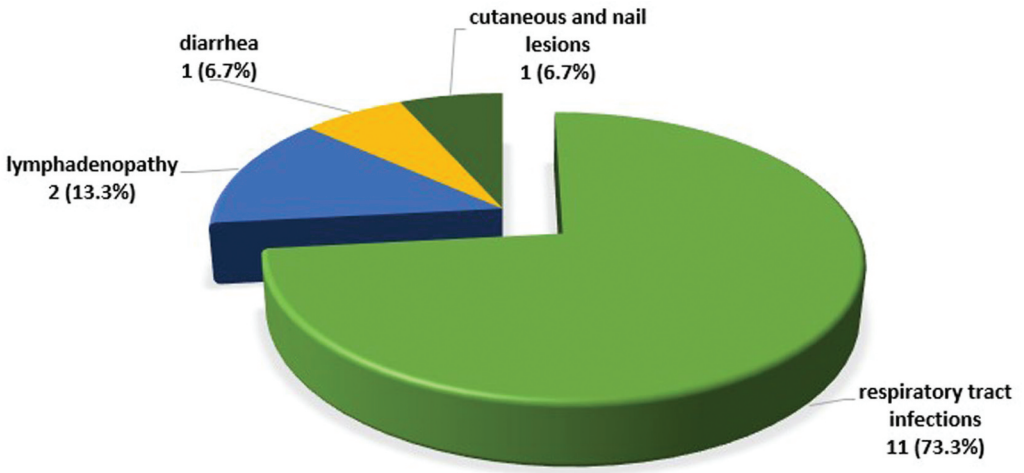

Figure 1. First clinical presentation of the study population.

Table 1. Demographic data of the study population and their analytical comparison between patients with *PIK3CD* mutations (APDS1) and those with *PIK3R1* mutations (APDS2).

Parameters	APDS1 patients (N = 6)	APDS2 patients (N = 9)	*P*-value
Vital status, number (%)	4 (66.7)	4 (44.4)	0.12
Alive	1 (16.7)	5 (55.6)-	
Dead	1 (16.7)		
Unknown			
Age	9.75 (8.85–32.00)	25.00 (16.50–32.00)	0.124
Median (IQR), years			
Sex, number (%)	4 (66.7)	6 (66.7)	1.000
Female	2 (33.3)	3 (33.3)	
Male			
Age at onset of symptoms	36.00 (26.25–48.00)	48.00 (15.00–72.00)	0.677
Median (IQR), month			
Age at diagnosis	72.00 (60.00–348.00)	168.00 (78.00–222.00)	0.677
Median (IQR), month			
Delay in diagnosis	42.00 (12.00–314.25)	84.00 (29.50–175.50)	0.859
Median (IQR), month			
Course of disease	74.50 (69.00–350.25)	236.00 (120.00–309.00)	0.409
Median (IQR), month			
Follow up period	48.00 (24.00–48.00)	198.00 (102.75–249.00)	0.077
Median (IQR), month			
Positive parental consanguinity	2 (33.3)	2 (22.2)	-
Number (%)			
Positive family history	5 (83.3)	0 (0)	0.002*
Number (%)			

IQR, interquartile range 25–75%.
**P*-value<0.05 is statistically significant.

Clinical manifestations

Among the total study population, recurrent respiratory tract infections (sinusitis, otitis media and pneumonia) were the most common clinical manifestation (86.7%) followed by lymphoproliferation (53.3%) and autoimmunity (26.7%). Respiratory tract infections (66.7%), autoimmunity and infections with herpes virus family (both 50%), and lymphoproliferation (33.3%) were the most common clinical manifestations in the APDS1 group, while respiratory tract infections (88.9%), lymphoproliferation (66.7%) and chronic diarrhea (33.3%) were the frequent clinical features in the APDS2 group (Figure 2). Interestingly, vaccination complications were observed in two of our studied patients: one APDS1 patient with poliomyelitis following oral polio vaccination who now suffers from paralysis and movement defect in her left arm and one APDS2 patients with fatal disseminated *Bacillus Calmette-Guérin* (BCG) infection following BCG vaccination. There was no statistically significant difference in terms of clinical features between the two groups; however, the severe medical conditions were significantly more frequent in the APDS2 group in comparison to the APDS1 group (frequency: 100% versus 33.3%, $P = .011$). The comparison of clinical features between the two groups is demonstrated in Table 2. Moreover, a summary of complicated organs in the study population is indicated in Table S1.

Laboratory data

As with the clinical manifestations, we found no significant differences in the immunological variables between the two groups ($P > .05$) (data not shown). However, the median (IQR)

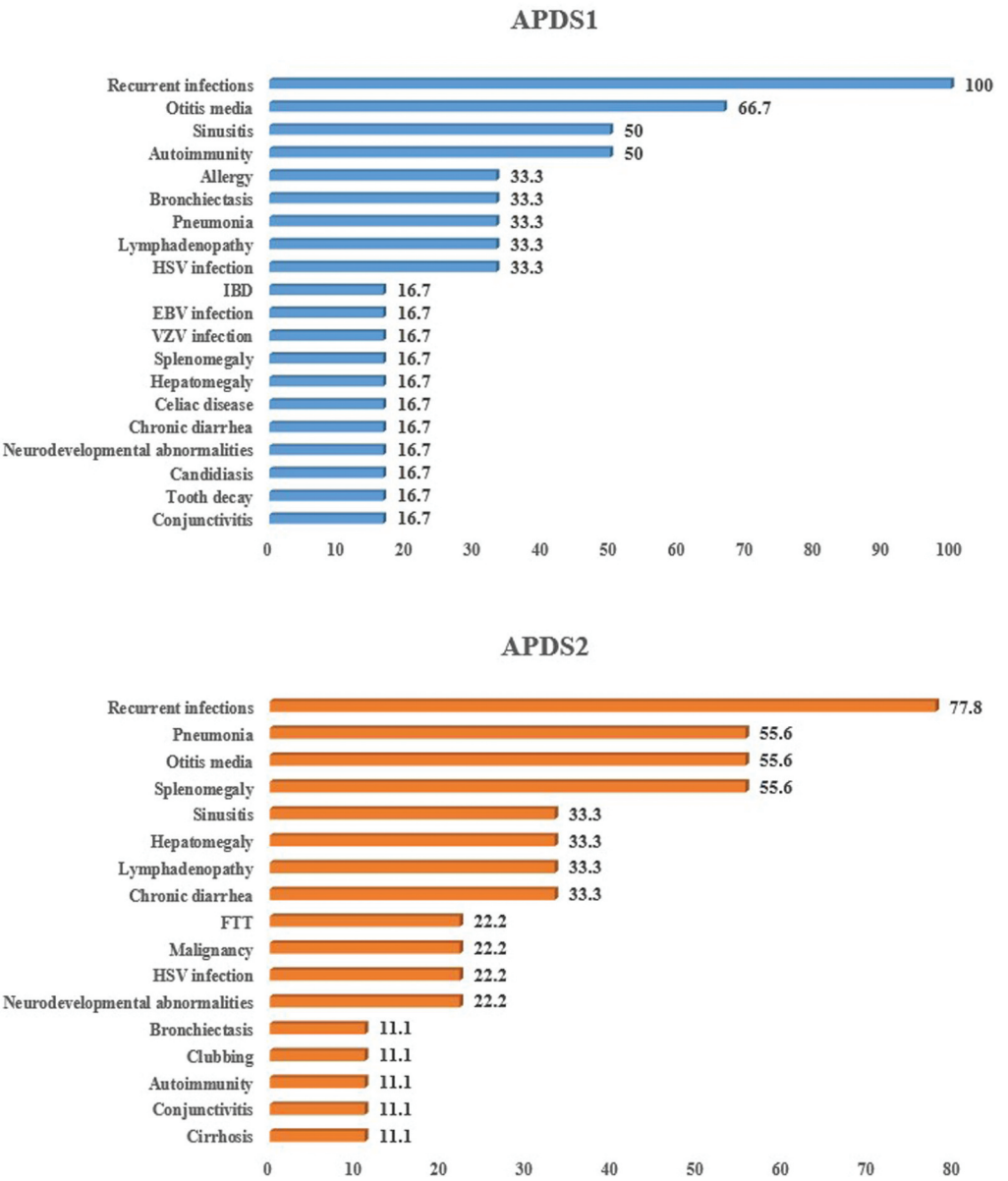

Figure 2. Comparison of clinical features in APDS1 and APDS2 patients. FTT: failure to thrive; IBD: inflammatory bowel disease; HSV, herpes simplex virus; EBV, Epstein–Barr virus; VZV, varicella-zoster virus.

serum level of IgM in APDS2 patients trended higher in comparison with the APDS1 group [220.0 (62.5–542.5) mg/dl versus 72.0 (5.1–95.25) mg/dl, $P = .087$]. We divided the patients' Ig profiles into four groups based on the criteria proposed by the European Society for Immunodeficiencies (ESID) (http://esid.org/Working-Parties/Registry/Diagnosis-criteria):

1. Hyper IgM (HIgM) characterized by normal or increased serum level of IgM along with decreased serum level of IgA and IgG; 2. Hypogammaglobulinemia characterized by decreased

Table 2. Clinical manifestations of the study population and their analytical comparison between patients with *PIK3CD* mutations (APDS1) and those with *PIK3R1* mutations (APDS2).

Parameters N (%)	APDS1 patients (N = 6)	APDS2 patients (N = 9)	*P*-value
Recurrent infections	6 (100.0)	7 (77.8)	0.486
Sinusitis	3 (50.0)	3 (33.3)	0.622
Otitis media	4 (66.7)	5 (55.6)	1.000
Pneumonia	2 (33.3)	5 (55.6)	0.608
Bronchiectasis	2 (33.3)	1 (11.1)	0.525
Conjunctivitis	1 (16.7)	1 (11.1)	1.000
Tooth decay	1 (16.7)	0 (0)	0.400
Candidiasis	1 (16.7)	0 (0)	0.400
Allergy	2 (33.3)	0 (0)	0.143
Autoimmunity	3 (50.0)	1 (11.1)	0.235
Clubbing	0 (0)	1 (11.1)	1.000
FTT	0 (0)	2 (22.2)	0.486
Lymphoproliferation	2 (33.3)	6 (66.7)	0.315
Lymphadenopathy	2 (33.3)	3 (33.3)	1.000
Hepatomegaly	1 (16.7)	3 (33.3)	0.756
Splenomegaly	1 (16.7)	5 (55.6)	0.300
Herpes virus family infection	3 (50.0)	2 (22.2)	0.329
HSV infection	2 (33.3)	2 (22.2)	1.000
EBV infection	1 (16.7)	0 (0)	0.400
VZV infection	1 (16.7)	0 (0)	0.400-
CMV infection	0 (0)	0 (0)	
BCGosis	0 (0)	1 (11.1)	1.000
Poliomyelitis	1 (16.7)	0 (0)	0.400
Malignancy	0 (0)	2 (22.2)	0.486
Neurodevelopmental abnormalities	1 (16.7)	2 (22.2)	1.000
Chronic diarrhea	1 (16.7)	3 (33.3)	0.604
Cirrhosis	0 (0)	1 (11.1)	1.000
IBD	1 (16.7)	0 (0)	0.400
Celiac disease	1 (16.7)	0 (0)	0.400

N, number; FTT, failure to thrive; HSV, herpes simplex virus; EBV, Epstein–Barr virus; VZV, varicella-zoster virus; CMV, cytomegalovirus; BCGosis, *Bacillus Calmette-Guérin* infection; IBD, inflammatory bowel disease.
Autoimmunity included celiac disease, systemic lupus erythematosus (SLE), arthritis and IBD.
Speech defect and growth retardation were considered a neurodevelopmental defect.
**P*-value<0.05 is statistically significant.

serum levels of at least one of IgG, IgA or IgM serum levels; 3. Agammaglobulinemia characterized by serum IgG level below 200 mg/dl in infants aged <12 months and 500 mg/dl in children aged >12 months or normal IgG levels with IgA and IgM below 2 standard deviations (SD) and 4. IgA deficiency (IgAD) characterized by serum levels of IgG and IgM within the age- and sex-matched reference values but IgA below 2SD. Hypogammaglobulinemia, agammaglobulinemia and IgAD profiles were equally frequent among APDS1 group (all in two patients, 33.3%), while HIgM (five patients, 55.6%) followed by hypogammaglobulinemia (three patients, 33.3%) and IgAD (one patient, 11.1%) were the more common profiles among APDS2 patients. Median absolute counts of all lymphocyte subsets as well as the median of all Ig levels including IgG and IgA (except IgM) were lower in patients with APDS2 than APDS1 group, although these differences were not significant.

Genetic analysis

Mutation analysis was performed using the WES method. In both APDS groups, de novo mutations with autosomal dominant inheritance pattern were confirmed. Multiple cases were confirmed in two siblings with IgAD profile (P1 and P2). In APDS1 group, splice site

(50%, mainly c.1339 + 4 G > A in twin sisters) and missense (50%, c.3061 G > A in three patients from different kindreds) mutations were equally prevalent. C-lobe was the most affected domain followed by C2 and N-lobe regions (50%, 33.3% and 16.7%, respectively). Among the mutations found in the APDS2 group, splice site mutation (77.8%, mainly c.1425 + 1 G > A in P7, P9 and P15 from three different kindreds) was the most common mutation followed by a missense mutation (12.2%). Inter-SH2 domain was the most affected domain followed by BH and N-SH2 regions (66.7%, 22.2% and 11.1%, respectively). Detailed information regarding DNA sequence change, zygosity, inheritance, the affected domain, type of mutation, predicted severity and medical severity is shown in Table 3.

Discussion

This is the first cohort study of Iranian pediatric patients with APDS. We have reported demographic, clinical, immunological and genetic characteristics of patients diagnosed with APDS1 or APDS2 and compared them between these two groups of patients. In our recent systematic review on APDS patients, we compared various characteristics between APDS1 and APDS2 patients (Jamee, 2019). Herein, we report a comparison of these variables between 15 new pediatric APDS patients from the national PID registry. Respiratory tract infections were the most common clinical feature among both groups. HIgM was the most frequent Ig profile in the APDS2 group, while hypogammaglobulinemia, agammaglobulinemia and IgAD profiles had equal frequencies among APDS1 patients.

According to our findings, patients with more than 4 years of delay in diagnosis were significantly older than those with lower than 4 years of delay in diagnosis. This difference could be due to the novelty of APDS as it has been recognized less than a decade from its first description in 2013 (Angulo et al. 2013). Moreover, confirming this disorder needs genetic analysis, typically by WES, for a diagnosis as phenotypic similarities are present in several other monogenic disorders associated with humoral immunodeficiency. Although affected patients are being diagnosed more rapidly and in earlier stages of the disease compared to previous years, further studies and guidelines are required for a better and more precise comprehensive management of APDS, including defining when and if PI3Kδ inhibitor therapy is warranted.

In the present study, upper and lower respiratory tract infections accounted for more than half of the clinical features in both APDS1 and APDS2 groups. Most of our patients (73.3%) had respiratory tract infections as the first clinical presentation and all of these complications were present before the age of 7 years old. Eighty percent of our patients had experienced at least one episode of sinusitis, otitis media and/or pneumonia during their lifetime. Similarly, other studies have also reported recurrent respiratory tract infections as the prominent clinical manifestations both at the onset of disease (Jamee, 2019, Kracker et al. 2014; Takeda et al. 2017) and during disease among APDS1 (Angulo et al. 2013; Chiriaco et al. 2017; Coulter et al. 2017; Crank et al. 2014; Elgizouli et al. 2016; Florea et al. 2017; Hartman et al. 2015; Heurtier et al. 2017; Kracker et al. 2014, Lucas, 2014; Takeda et al. 2017; Wentink et al. 2017) and APDS2 (Deau et al. 2014; Elkaim et al. 2016; Lougaris et al. 2015, Lucas, 2014; Petrovski et al. 2016; Wentink et al. 2017) patients. Among our patients with recurrent respiratory tract infections, bronchiectasis was detected in three patients (20%), two with APDS1 and one with APDS2. Although all participating centers follow consensus medical indications for chest CT scanning, a lower

Table 3. Genetic data of the study population.

Patients' ID	Gene	Deleterious variants	Protein change	Zygosity	Inheritance	PMID reported/new patient	Method	Affected domain	Type of mutation	Prediction severity	Medical severity
P1	*PIK3CD*	c.1339 + 4 G > A	Splicing	Het	AD	New case	WES	C2	Splice site	Severe	Mild
P2	*PIK3CD*	c.1339 + 4 G > A	Splicing	Het	AD	New case	WES	C2	Splice site	Severe	Mild
P3	*PIK3CD*	c.3061 G > A	p. E1021K	Het	AD	27980538	WES	C-Lobe	Missense	Mild	Severe
P4	*PIK3CD*	c.3061 G > A	p. E1021K	Het	AD	New case	WES	C-Lobe	Missense	Severe	Mild
P5	*PIK3CD*	c.3061 G > A	p. E1021K	Het	AD	New case	WES	C-Lobe	Missense	Mild	Mild
P6	*PIK3CD*	c.2027 + 5 C > T	Splicing	Het	AD	New case	WES	N-Lobe	Splice site	Severe	Severe
P7	*PIK3R1*	c.1425 + 1 G > A	Splicing	Het	AD	29599784	WES	i-SH2	Splice site	Severe	Severe
P8	*PIK3R1*	c.1423_1425 + 9 delCAGGTGAGTTTT	Splicing	Het	AD	29599784	WES	i-SH2	Splice site	Severe	Severe
P9	*PIK3R1*	c.1425 + 1 G > A	Splicing	Het	AD	29599784	WES	i-SH2	Splice site	Severe	Severe
P10	*PIK3R1*	c.1558 T > G	p.S520A	Het	AD	New case	WES	i-SH2	Missense	Mild	Severe
P11	*PIK3R1*	c.1558 T > G	p.S520A	Het	AD	New case	WES	i-SH2	Missense	Mild	Severe
P12	*PIK3R1*	c.336 + 1 G > A	Splicing	Het	AD	New case	WES	BH	Splice site	Severe	Severe
P13	*PIK3R1*	c.336 + 2 T > G	Splicing	Het	AD	New case	WES	BH	Splice site	Severe	Severe
P14	*PIK3R1*	c.1020–8 C > G	Splicing	Het	AD	New case	WES	N-SH2	Splice site	Severe	Severe
P15	*PIK3R1*	c.1425 + 1 G > A	Splicing	Het	AD	29599784	WES	i-SH2	Splice site	Severe	Severe

ID, identification; P, patient; Het, heterozygote; AD, autosomal dominant; AR, autosomal recessive; WES, whole-exome sequencing; BH, breakpoint cluster region homology domain; ABD, Adaptor-binding domain; N-lobe, N-terminal lobe; C-lobe, C-terminal lobe; i-SH2, inter-SH2.

incidence of bronchiectasis in our cohort may reflect local differences in thresholds for performing imaging or the overall lower age of this pediatric cohort with shorter period to develop such complication. The patients with bronchiectasis had a significantly longer delay in diagnosis compared to those without structural lung damage. Complications of later diagnosis including permanent hearing loss from recurrent otitis media and bronchiectasis from recurrent pneumonia have previously been reported (Angulo et al. 2013; Coulter et al. 2017; Heurtier et al. 2017; Kracker et al. 2014, Lucas, 2014; Mettman et al. 2017; Nademi et al. 2017; Rae et al. 2017; Takeda et al. 2017; Wentink et al. 2017). Therefore, recurrent respiratory tract infections should be considered as major complications in APDS patients and our findings consistent with the previous reports highlight the importance of earlier diagnosis of the underlying PID and timely initiation of treatment before irrecoverable complications.

Non-neoplastic lymphoproliferation (lymphadenopathy, hepatomegaly and splenomegaly) was the second most common clinical manifestation among our APDS patients, especially the APDS2 group. We found no significant difference in lymphoproliferation disorders between the two groups, contrary to findings of Jamee *et al.* (Jamee, 2019) who reported a significant difference in lymphadenopathy and hepatomegaly between APDS1 and APDS2 patients. Also in contrast to the findings of Coulter *et al.* who reported 75% non-neoplastic lymphoproliferation disorders among APDS1 patients (Coulter et al. 2017), only two (33.3%) of our APDS1 patients had lymphoproliferation disorders: one had lymphadenopathy and the other had cervical lymphadenopathy following Epstein–Barr virus (EBV) infection along with hepatosplenomegaly. The lower percentage of lymphoproliferation disorders in our study population in comparison to previous studies (Angulo et al. 2013, Lucas, 2014, Lucas, 2014) could partly be due to environmental factors and underlying viral infections particularly EBV and cytomegalovirus (CMV) infections. Moreover, younger age of patients in our cohort resemble the findings of Maccari *et al.* (Maccari et al. 2018) demonstrating an evolving picture of clinical manifestations starting with respiratory infections presentation and lymphadenopathy occurring in later stages with enteropathy and cytopenias. Nevertheless, the role of other factors in the predisposition of APDS patients to lymphoproliferation such as genetic and epigenetic factors needs to be further studied.

Pathogenic mutations in *PIK3CD* and *PIK3R1* appear to have oncogenic potential as the PI3K/Akt/mTOR signaling pathway plays a pivotal role in controlling the proliferation and survival of tumor cells (Majchrzak et al. 2014). Hyperactivity within this pathway could predispose to the development of malignancy in APDS patients. Malignancies, particularly lymphomas, are a life-threatening feature among APDS patients and have a wide range of onset ranging from 1 to 40 years old with a high incidence of 10–30% (Cansever et al. 2020; Coulter et al. 2017; Crank et al. 2014; Elkaim et al. 2016; Florea et al. 2017; Heurtier et al. 2017; Kracker et al. 2014, Lucas, 2014, Lucas, 2014). In such cases, almost 50% of lymphoma development is associated with previous herpes virus family especially EBV infection (Jamee, 2019, Lucas, 2014). In our study population, two cases had malignancies: one of them had ovarian cystadenoma and the other had large cell lymphoma. Physicians should be aware of this complication and malignancy work-up should be performed in the periodic follow-up of the patients for earlier diagnosis and management of them especially in patients with chronic and non-resolving EBV infection.

For the first time, we report an APDS1 patient (P3 in Table 3) who had poliomyelitis following oral polio vaccination (OPV) with a resultant paralysis and movement defect in her left arm. In our previous study, we have reported a considerable number of paralytic polio infection as a result of OPV in μ heavy chain-deficient patients (Yazdani et al. 2019). Humoral immunity seems to be the predominant immunity against enteroviruses, thus patients with major B-cell dysfunction are at increased risk for poliomyelitis (Yazdani et al. 2019). Of note, our affected APDS1 patient had an agammaglobulinemia Ig profile which may be the cause of polio infection. Nevertheless, the exact pathogenesis is unclear and needs to be further studied.

We have evaluated medical severity in APDS patients and compared it between APDS1 and APDS2 groups. According to our findings, APDS2 patients had significantly more severe medical conditions than APDS1 patients, but it is interesting that there were no significant differences in the frequency of clinical features between these two groups of patients, perhaps suggesting discreet complications of PIK3-AKT-mTOR in non-immune tissues. However, further studies with a larger number of patients are required to define the association between clinical, genetic, and molecular defects with the severity of APDS and help identify severe cases more rapidly and in earlier stages of the disease for optimal treatment, including potentially HSCT (Jamee, 2019).

PI3K signaling is required for the proper Ig isotype class switching in primary B cells. PI3K actively suppresses class switch recombination (CSR) through the inactivation of FOXO transcription factors leading to the HIgM condition (Omori et al. 2006). In our study, five APDS2 patients had a HIgM-like phenotype, while none of the APDS1 patients had this immunological profile. This difference was statistically significantly similar to the findings of our recent systematic review in which HIgM immunophenotype was more prevalent in the APDS2 group in comparison to the APDS1 group (Jamee, 2019). Thus, APDS should be considered as a differential diagnosis of HIgM syndrome (e.g. AICDA and UNG deficiencies) due to similarities in clinical and immunological characteristics between these two PIDs. Other Ig profiles among our patients were hypogammaglobulinemia, agammaglobulinemia and IgAD profiles. Other studies have also reported these profiles as well as normal Ig levels among APDS1 and APDS2 patients (Coulter et al. 2017; Deau et al. 2014; Elkaim et al. 2016, Jamee, 2019, Lucas, 2014). Our findings along with the others suggest that the Ig profile has a wide spectrum in APDS patients and that it cannot be a proper indicator of APDS mutation type.

The most-reported mutation in APDS1 is a missense mutation (c.3061 G > A p. E1021K) affecting the C-lobe region of the *PIK3CD* gene. Other rare mutations are missense mutations at ABD (c.241 G > A p. E81K), the linker between RBD and ABD (c.371 G > A p. G124D), C2 (c.1002 C > A p. N334K, c.1213 C > T p. R405C and c.1246 T > C p. C416R), Helical (c.1570 T > A p. Y524N; c.1573 G > A p. E525K and c.1574 A > C p. E525A) and C-Lobe (c.2784 C > T p. R929C, c.3061 G > A p. E1021K and c.3074 A > G p. E1025G) regions of *PIK3CD* (Angulo et al. 2013; Baleydier et al. 2019; Cansever et al. 2020; Chiriaco et al. 2017; Coulter et al. 2017; Crank et al. 2014; Edwards et al. 2019; Elgizouli et al. 2016; Elkaim et al. 2016; Florea et al. 2017; Hartman et al. 2015; Heurtier et al. 2017; Hong et al. 2019; Kracker et al. 2014, Lucas, 2014; Luo et al. 2018; Mettman et al. 2017; Nademi et al. 2017; Okano et al. 2019; Rae et al. 2017; Ruiz-García et al. 2018; Takeda et al. 2017; Wentink et al. 2017). Three of our APDS1 patients had novel mutations: two splice site mutations affecting C2 (c.1339 + 4 G > A) and N-lobe (c.2027 + 5 C > T) regions. Of note, these novel

mutations have not confirmed by functional assays yet and these findings are solely based on in silico predictions and clinical phenotyping. Further lymphocyte subset analysis including transitional B cells and senescent T cells could be conducted in future to support this finding. On the other hand, most of the reported mutations in *PIK3R1* are splice site mutations at the splice donor or acceptor sites of intron 10 affecting the inter-SH2 domain of the gene and leading to the skipping of exon 10 and a truncated $p85\alpha^{\Delta 434-475}$ [c.1299 + 1 G > A, c.1300–1 G > C, c.1425–1 G > C, c.1425 + 1 G>(A, C, T), c.1418_1425 + 1del, c.1425 + 2 T>(G, A), c.1425 + 2 A > T, c.1425 + 2,3delTG and c.1423_1425 + 9delCAGGTGAGTTTT], which hyperactivates PIK3δ through the loss of inhibitory contact between the regulatory and catalytic subunits (Coulter et al. 2017; Deau et al. 2014; Dominguez-Pinilla et al. 2018; Elkaim et al. 2016; Kuhlen et al. 2016; Lougaris et al. 2015, Lucas, 2014; Martínez-Saavedra et al. 2016; Nademi et al. 2017; Olbrich et al. 2016; Petrovski et al. 2016; Wentink et al. 2017).

The other less reported mutation is a missense mutation affecting the inter-SH2 domain (c.1692 C > G p. N564K) (Elkaim et al. 2016; Wentink et al. 2017). In the current study, we found four novel mutations that are three splice site mutations: c.336 + 1 G > A and c.336 + 2 T > G with autosomal dominant inheritance pattern affecting the breakpoint cluster region homology domain (BH), c.1020–8 C > G with autosomal recessive inheritance pattern affecting the N-SH2 domain as well as a missense mutation (c.1558 T > G) with autosomal dominant inheritance pattern affecting the inter-SH2 domain. Our findings indicate that in APDS patients, other domains of *PIK3CD* and *PIK3R1* could be affected as well. Increasing our knowledge about APDS with proper diagnostic criteria could lead to earlier diagnosis of the patients and conducting molecular analysis in the affected patients could help to better understand the probable correlations between genetic mutations and disease severity, suggesting the timely application of the appropriate treatment.

In our study, five APDS2 and one APDS1 patients died: four of the APDS2 patients died because of chronic lung infection and the remaining one due to disseminated *Bacillus Calmette-Guérin* infection (BCGosis); while the APDS1 patient died because of lymphoproliferative disease. Other reported causes of death among APDS patients are lymphoma, EBV lymphoproliferative disease, large-bowel perforation, multi-organ failure, septic shock and respiratory failure due to chronic lung infection (Coulter et al. 2017; Elkaim et al. 2016; Kracker et al. 2014; Takeda et al. 2017). The important fact is that lymphoma and chronic lung infections are the leading causes of death among APDS patients. Hence, timely diagnosis and management of these complications in APDS patients, mainly for *PI3KR1* mutations is vital and could save the patients' lives and improve their quality of life.

Conclusion

APDS is a recently recognized combined PID with a variety of clinical features. Respiratory tract infections are commonly the most important complication and they can be fatal by leaving catastrophic damage to the structure of lungs. Hence, physicians should be aware of its significance and further work-up of patients with recurrent respiratory tract infections especially in those with lymphoproliferation. Moreover, the identification of defective mutations and their association with disease severity using a gene-specific database could be helpful in the timely application of appropriate management and patients' survival.

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The authors declare no conflict of interest.

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