Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
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ORIGINAL ARTICLE

OPEN ACCESS

Clinical and immunological assessment of APDS2 with features of the SHORT syndrome related to a novel mutation in *PIK3R1* with reduced penetrance

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Received 22 September 2021; Accepted 15 December 2021
Available online 1 July 2022

KEYWORDS
APDS2;
immune dysregulation;
immunodeficiency;
PIK3R1;
SHORT syndrome

Abstract
Monoallelic loss-of-function (LOF) mutations in the phosphatidylinositol 3-kinase (*PIK3R1*) gene affecting the inter-Src homology 2 domain of the p85α regulatory subunit of phosphoinositide-3-kinase δ (PI3Kδ) cause the activated PI3K δ syndrome (APDS2). APDS2 is defined as a primary antibody deficiency, developmental abnormalities within the B and T lymph cell compartments, and immune dysregulation. The genetic defect of APDS2 is shared with that of the SHORT syndrome, characterized by short stature, joint hyperextensibility, ocular depression, Rieger anomaly, and delayed tooth eruption. LOF variants in an intronic splice site (c.1425+1G.C/A/T) in the *PI3KR1* gene have been identified in patients affected with both APDS2 and SHORT syndrome. Herein, we report a novel c.1644-1648del (p.Asp548Glufs*6) variant in a pediatric patient with the APDS2-related immunodeficiency, who presents with mild phenotypic features of the SHORT syndrome, congenital chest wall deformity, and IgE-mediated food allergy. The same variant was also identified in the patient's hitherto asymptomatic mother, implicating an incomplete penetrance. Regular monitoring by a multidisciplinary team under the pediatric clinical immunologist's supervision to implement appropriate diagnostic procedures and treatment modalities is of paramount importance. Further studies are required to better define the genotype-phenotype correlation in patients with the *PIK3R1* gene mutations and to better delineate the mutual relationship between APDS2 and the SHORT syndrome.

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https://doi.org/10.15586/aei.v50i4.510

Introduction

The SHORT syndrome is a rare genetic multisystemic condition, delineated by the clinical phenotype and defined by its acronym, including short stature, inguinal hernia and/or hyperextensibility of joints, ocular depression, Rieger anomaly of the ocular anterior chamber, and delayed teeth eruption.[1] Marked heterogeneity of clinical features has been noted in affected patients and ascribed to the syndrome, such as facial dysmorphology with a triangular face, prominent forehead, deep-set eyes, mildly hypoplastic midface and nasal alae, maxillary hypoplasia with a small chin, downturned mouth, and low-set ears. This syndromic condition is typically accompanied by lipodystrophy with a lack of adipose tissue situated at the face, arms, and chest, with wrinkled and thin, translucent skin resulting in progeroid appearance. An ocular defect caused by anterior segment dysgenesis characterized by anterior chamber deformity and atrophy of the iris stroma, displacement of the pupil (corectopia), and coloboma of the iris also known as Rieger anomaly that, in turn, can lead to the development of glaucoma.[2,3] A case of the SHORT syndrome with hyperopia and astigmatism associated with poor visual acuity has also been described.[2] Dental abnormalities also belong to the characteristic phenotypic features and comprise delayed teeth eruption, missing or decreased number of teeth (adontia or hypodontia, respectively), small teeth (microdontia)[4] and overcrowded, irregular teeth.[5] An extended SHORT syndrome phenotype also comprises polycystic ovary syndrome and diffuse thyroid disease,[5] delayed bone age, cryptorchid testes,[6] deviated nasal septum,[6] short toes, phalanges of fingers, and metacarpals.[5,7] A hallmark of the syndrome is an impaired carbohydrate metabolism with insulin resistance leading to an early-onset type 2 diabetes usually during the second decade of life and lipoatrophy, without dyslipidemia and fatty liver disease.[8] Delayed speech development, sensorineural hearing loss requiring hearing aids, and mild cognitive delay have also been reported in patients with the SHORT syndrome, but intelligence is usually within normal range and most children can have normal educational achievements.[1]

The exact prevalence of the SHORT syndrome is not known, but it has been estimated to occur with a frequency of fewer than 1:1.000,000 cases, and several dozen of cases have been reported so far in the medical literature. The syndrome has been registered in the Orphan diseases database as OMIM 269880 / ORPHA:3163 (4). The mode of inheritance is autosomal dominant. Heterozygous loss-of-function (LOF) and gain-of-function mutations in the phosphatidyl inositol-3-kinase class 1A (*PIK3R1*) gene encoding for p85α regulatory subunit of phosphoinositide 3-kinase (PI3Kδ) cause an impaired activation of the AKT-mTOR signaling pathway, which is involved in cellular proliferation, growth, and metabolism. Disruption of the PI3K-AKT-mTOR regulatory mechanism causes a combined primary immunodeficiency disease referred to as activated PI3Kδ syndrome (APDS2). [9,10] Heterozygous LOF mutations in *PIK3R1* also constitute the genetic background of the SHORT syndrome; however, the coexistence of APDS2 and the SHORT syndrome is rare with hitherto merely four cases described[11-13] whose clinical and immunological assessment is summarized in Table 1. Furthermore, the symptomatology, such as poor growth, short stature, developmental delay, and microcephaly, noted with high incidence in childhood-onset combined immunodeficiency related to APDS2 does not comply with a constellation of syndromic features peculiar to the SHORT syndrome.[14-17]

In APDS2, also known as p85α-activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI-R1), the defects in PI3K signaling pathways are leading to immunodeficiency and immune dysregulation, with B and T cell lymphopenia, lymphocyte maturational and functional defects, and antibody deficiency, frequently presented as the hyper-IgM immunoglobulin profile.[18-20] The individual patient's immunophenotype is determined by the mutation in *PIK3R1*, as mono-allelic LOF mutations cause activation of the PI3K-AKT-mTOR pathway with defects in lymphocyte development and variable immunoglobulin deficiency and have been noted in patients with both APDS2 and SHORT syndrome,[11-13] whereas biallelic LOF mutations impair the PI3K pathway, resulting in more profound abnormalities in B cells and agammaglobulinemia.[21]

To expand the clinical and immunological phenotype of an extremely rare association of APDS2 and mild phenotypic features of the SHORT syndrome in a pediatric patient who shows unique and previously unreported dysmorphology features with a congenital deformity of the chest, a stellate pattern of the iris, accompanied by IgE-mediated cow's milk allergy, and antibody deficiency, in the context of a frameshift mutation in *PIK3R1* with reduced penetrance.

Case Description

A 5-month-old boy was referred to our pulmonology, allergy, and clinical immunology unit of the Pediatric University Hospital due to bronchiolitis and pneumonia.

He was born as the second child of a young, healthy, nonconsanguineous couple, preterm, at 36 weeks gestational age (WGA), by natural forces, in a good general condition, with an Apgar score of 10. His morphometric parameters at birth were the following: the birth weight 2480 g (10–25 percentile), body length 47 cm (25–50 percentile), and head circumference 31 cm (3–10 percentile), all percentile values corrected for prematurity between 35 and 38 WGA. At birth, a congenital chest wall deformity (CCWD) pectus excavatum was noted. The family history was contributory, the maternal brother and cousin also suffered from the same CCWD. In the perinatal period, he presented symptoms of cow's milk intolerance with regurgitations, flatulence, and abdominal colic pain. Due to those symptoms, a cow's milk-free diet based on hydrolyzed whey formula was recommended. At the age of 4 weeks, he was diagnosed at the cardiology unit of our hospital and the compression of the heart by a depressed chest wall was excluded. The reparative thoracosurgery was projected to take place at the age of 6 years. A heart murmur was also discovered upon examination, which raised suspicion of a congenital heart defect. The murmur was classified as an innocent murmur due to a false

Table 1 Summary of clinical, immunological and genetic assessment APDS2 and SHORT patients

Reference	Age at diagnosis	PIK3R1 variant	APDS2 immunodeficiency		SHORT phenotype
			Symptomatology	Laboratory assessment	Symptomatology
Petrovski et al.[11]	5 years	c.1425+ 1G>A splice site mutation	- Otitis media - Dacryocystitis - Pneumonias	Aged 5 years - low IgG and IgA, normal IgM - Low transitional, memory and switched-memory B cell numbers - Proliferation assays normal	- Facial dysmorphism - Micrognatia - Deep-set eyes - Short stature - Delayed permanent teeth eruption - Joint laxity - Lipodystrophy
Ramirez et al.[12]	17 years	c.1425+ 1G>C splice site mutation	- Chronic rhinitis - Mild intermittent asthma - Recurrent upper respiratory tract infections - Recurrent otitis media - Hordeolums - *Clostridium difficile* colitis - Vaginal candidiasis	Aged 17 years - low IgG and IgA, normal IgM - low B cell numbers - Vaccination response to *Haemophilus influenzae, Streptococcus.pneumoniae*, and *Diphtheria* normal, absent to *Tetanus* - Proliferation assays normal	- Facial dysmorphism - Short stature - Poor weight gain - Delayed permanent teeth eruption - Joint laxity - Chiari I malformation
Bravo Garcia-Morato et al.[13]	10 years	c.1425+ 1G>A splice site mutation	- Recurrent otitis media - Recurrent respiratory tract infections - Conjunctivitis - Cellulitis - Lymphadenopathy	Aged 10 years - low IgA, normal IgM and IgG - %B transitional cells high - Vaccination response to *Tetanus* and *Diphtheria* toxoids normal - Proliferation assays moderately reduced	- Translucent skin - Facial dysmorphism - Mild conductive hearing loss - Joint laxity - Mitral stenosis
Bravo Garcia-Morato et al.[13]	26 years	c.1425+ 1G>T splice site mutation	- Recurrent upper respiratory tract infections - Recurrent otitis media - *Staphylococcus aureus*, herpetic and fungal skin infections - Recurrent conjunctivitis and dacryocystitis - *Giardia* and *Cryptosporidium* infections - Lymphadenopathy, hepatosplenomegaly - Hodgkin lymphoma - Non-Hodgkin lymphoma	Aged 23 months - low IgG and IgA, high IgM - Proliferation assays moderately reduced	- Poor growth and weight gain - Facial dysmorphism - Delayed permanent teeth eruption - Mixed hearing loss - Dysplastic mitral valve and aortic valve insufficiency
Szczawińska-Popłonyk et al. (current report)	20 months	c.1644-1648del frameshift mutation	- Chronic rhinitis - Recurrent conjuctivitis - Recurrent respiratory tract infections RSV, Rhinovirus, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa* - Food allergy	Aged 18 months - low IgG, IgA, and IgM Aged 3 years - IgG (on IVIg) and IgA normal - low IgM - Proliferation assays normal - low numbers of follicular and regulatory Th cells	- Funnel-shaped chest - Facial dysmorphism - Deep-set eyes - Stellate iris pattern - Cryptorchid testis - Linea alba hernia - Lipodystrophy - Mild growth delay

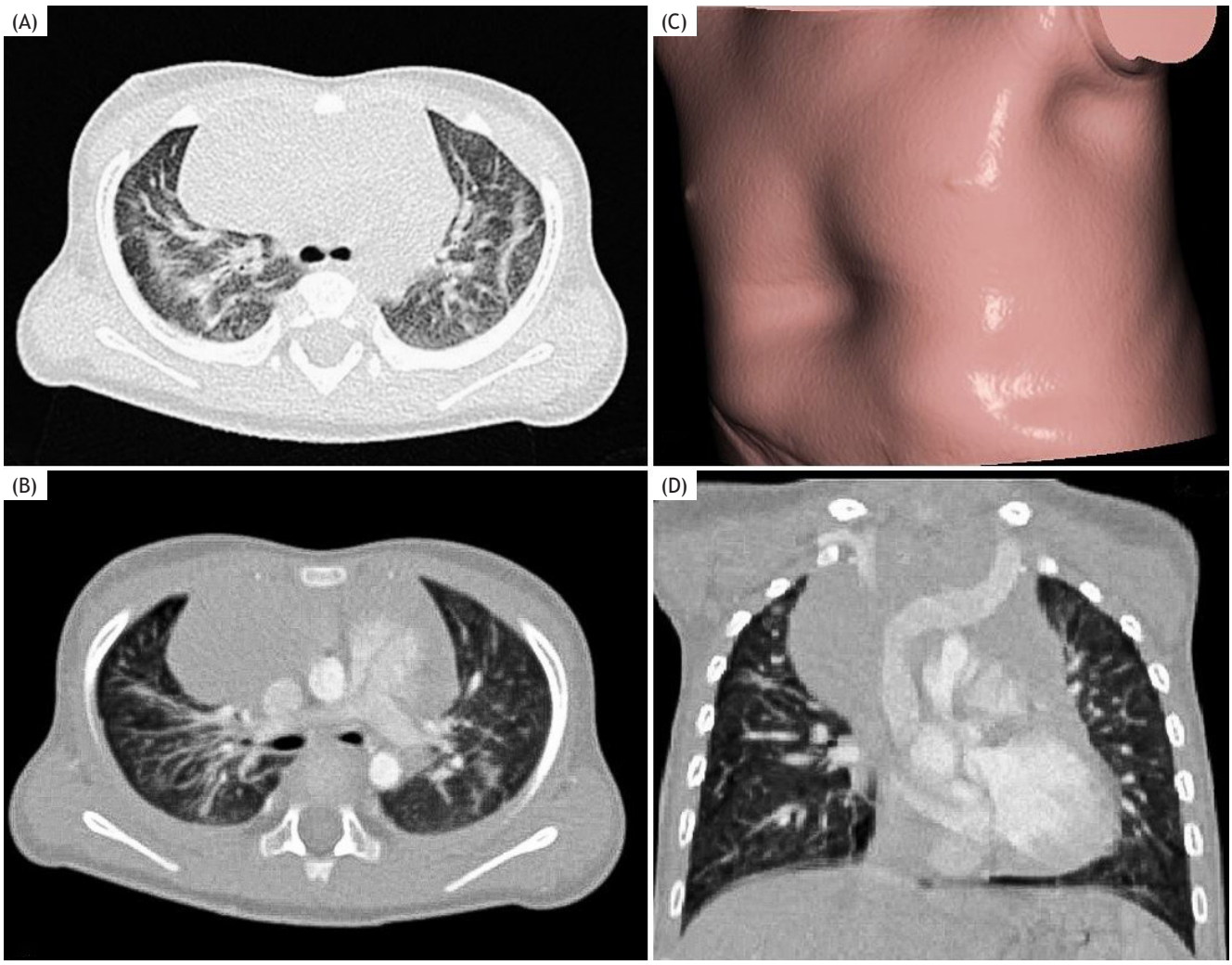

Figure 1 Thoracic HRCT imaging with contrast medium. (A) Axial view, pulmonary window. Bilateral fibrotic strands and mosaic perfusion in upper lobes of the lungs; (B) Axial view, pulmonary window. Fibrotic strands and mosaic perfusion in LS8 and LS9; (C) Volume rendered technique (VRT) showing chest deformity (pectus excavatum); (D) Coronal view. Chest deformity with modeling of the right atrium and right ventricle of the heart by a low part of the sternum.

tendon in the left ventricle, assessed based on echocardiography. Neurological assessment was also performed at that time because of the asymmetric position of the body and impaired central motor coordination was diagnosed on physical examination, with a slight asymmetry of brain lateral ventricles in transfontanellar ultrasonography. At the age of five months, a chest X-ray showed streaked parenchymal infiltrates in the upper lobe and the central parts of the right and left lung. Subsequently, throughout the 3 years, the patient achieved all psychomotor developmental milestones and the rates of gaining weight and height were corresponding with the age-matched values between 10 and 50, and between 3 and 25 percentiles, respectively. Since infancy, he also presented linea alba hernia and cryptorchid testis, and the reparative operations of the hernia and orchidopexy were performed at the age of 3.

The child received all live attenuated (BCG after birth and measles/mumps/rubella in the 13th month of life) and inactivated vaccines (against poliomyelitis, pertussis, tetanus, and a 10-valent polysaccharide conjugated pneumococcal vaccine), without adverse effects following immunization (AEFI).

Since the first episode of pneumonia at the age of 5 months, he developed recurrent obstructive bronchitis, which was treated with inhaled bronchodilators and oral antibiotics. At the age of 13 months, a thoracic high-resolution computed tomography (HRCT) showed bilateral fibrotic strands in upper lobes and segments of the left lower lobe, accompanied by mosaic perfusion and large thymus with its right lobe reaching the diaphragm (Figure 1A and 1B). The CCWD, in the form of pectus excavatum, the low part of the sternal body, and the xiphoid process was shaping the right atrium and right ventricle of the heart. The anteroposterior dimensions of the chest were as follows: 2,9, 5,0, and 4,8 cm, measured at levels of the superior thoracic aperture, in the middle part of the chest, at Th4, and in the lower part of the chest, at Th6, respectively (Figure 1C and 1D). The ECG did not show features of ischemia, and echocardiography revealed normal anatomical and functional parameters of the heart muscle. The

abdominal ultrasound examination did not show organomegaly, lymphadenopathy, or nephrocalcinosis.

At the age of 18 months, the laboratory workup showed immunoglobulin isotypes G, M, and A below the age-matched reference values, and hence, immunoglobulin replacement therapy (IgRT) was initiated, with a good clinical effect and alleviation of recurrent respiratory symptoms. Allergen-specific IgE (asIgE) to cow's milk antigens, β-lactoglobulin, α-lactoalbumin, casein, and cow's serum albumin were positive, and asIgE levels were corresponding with 3, 2, 1, and 1 class, respectively. At the age of three, he developed recurrent bronchitis, caused by viral infections of the respiratory tract and showed a poor response to inhaled bronchodilators and corticosteroids. Consequently, antimicrobial prophylaxis with azithromycin on an alternate-day regimen was initiated. The results of laboratory tests, including hematology, biochemistry, immunology, and microbiology, are summarized in Table 2. Relevant data from the episode of the patient's care (Timeline) are displayed in Figure 2.

At the age of 20 months, he was examined by a clinical geneticist, who found mild facial features of the SHORT syndrome but ascertained deep-set eyes, a particular stellate pattern of the iris, pectus excavatum, as well as dysplastic nails in hands and feet. Genetic testing included a standard cytogenetic analysis, which showed a normal male karyotype and Multiplex Ligation-dependent Probe Amplification testing for the detection of selected most frequent microdeletions (1p36, 2p16, 3q29, 9q22.3, 15q24, 17q21, 22q13/Phelan-McDermid syndrome, 5p15/Cri-du-Chat syndrome, 22q11.21/DiGeorge syndrome, 10p13-p14/DiGeorge syndrome-2, 8q24.11-q24.13/Langer-Giedion syndrome, 17p13.3/Miller-Dieker syndrome), which also proved normal. The next-generation sequencing analysis including deletion/duplication testing of the 207 genes (Invitae Primary Immunodeficiency Panel) revealed a novel heterozygous LOF pathogenic variant c.1644-1648del (p.Asp548Glufs*6) in the exon 13 in the *PIK3R1* gene. This finding was subsequently confirmed by Sanger sequencing as shown in Figure 3. The sequence change creates a premature translational stop signal in the *PIK3R1* gene and is expected to result in an absent or disrupted protein product. This newly identified variant has not been reported in the literature of individuals with PIK3R1-related disorders. This result might be consistent with a clinical diagnosis of autosomal dominant PIK3R1-related condition, activated PI3Kδ syndrome, and also with the SHORT syndrome, albeit coexisting phenotypic features of both APDS2 and the SHORT syndrome is exceptional.[11-13] The variant was also found in the patient's mother, who is a healthy individual, not affected with any comorbidities. She did not hitherto suffer from recurrent infections and did not present autoimmune, atopic, or lymphoproliferative diseases appearing in APDS2. She does not show ocular or facial dysmorphic features characteristic of the SHORT syndrome.

Discussion

The combination of APDS2 and SHORT syndrome has been described in medical literature only in four cases so far,[11-13] suggesting a rarity of this clinical phenotypic and genetic association. In the patients reported, a common genetic background with heterozygous, pathogenic LOF variants in an intronic splice site in the *PIK3R1* gene, c.1425+1G>A/C/T have been identified. These PIK3R1-related genotypes have been extensively detected in patients with APDS2 and hyperactivation of the PI3K-AKT-mTOR signaling pathway.[19,22,23] Whereas APDS2 and the SHORT syndrome are considered as two independent phenotypic and genetic conditions, a unifying pathway and common genetic background with c.1425+1G>A/C/T variants have been identified in patients sharing both immunological and non-immunological features of both conditions. It needs to be highlighted that in the presented patient, a novel heterozygous pathogenic LOF c.1644-1648del (p.Asp548Glufs*6) mutation in the *PIK3R1* gene. This sequence change creates a premature translational stop signal in *PIK3R1* gene and is expected to result in the absence or in a disrupted protein product. This variant is associated with combined features of the two PIK3R1-related clinical conditions, APDS2 and SHORT syndrome. To date, this variant has not been reported in the literature in individuals with PIK3R1-related conditions. The exact pathophysiological mechanisms linking both syndromes and their interrelations have not been elucidated so far, albeit our study shows a more extended genotype of combined APDS2-SHORT phenotype. This might also imply a need for further delineation and definition of both conditions. It is worth noting, that in APDS2, short stature has been reported in as many as 45% of affected patients,[23] likewise, syndromic features such as short stature, and delayed speech development were reported in the ESID survey of the APDS2 cohort.[24] Furthermore, cognitive impairment, short stature, and microcephaly have been described in APDS2 patients,[14,15,23] adding clinical features to the APDS phenotype, such as joint hyperextensibility, increased glucose level, and polycystic kidney disease in the latter report.[24] Certainly, poor growth, failure to thrive, and neurodevelopmental delay may also arise from severe immunodeficiency, chronic infections, immune dysregulation, and lymphoproliferative complications in APDS2, but also may result from an overlapping phenotype with the SHORT syndrome. Therefore, we might presume that a combination of APDS2 with a phenotypically incomplete SHORT syndrome is not infrequent.

An activated PI3Kδ syndrome is characterized by a complex immunophenotype with combined immunodeficiency and immune dysregulation, with a broad range of developmental and functional abnormalities within B and T lymph cell compartments. Progressive B-cell lymphopenia, reduced naïve mature, and switched and nonswitched memory B cells along with increased numbers of transitional B cells are accompanied by low numbers of naïve T cells and increased effector memory T cells and terminally differentiated effector memory cytotoxic T cells.[25-27] In our patient with a novel c.1644-1648del (p.Asp548Glufs*6) variant in *PIK3R1*, we observe APDS2-related immunodeficiency with low IgG and IgM serum levels, which require IgRT because of the chronic early-life lung damage and susceptibility to bacterial and viral infections of the respiratory tract.[28,29] Whereas the constitutive activation of the PI3K-AKT-mTOR signaling pathway is directly involved in the oncogenic process and APDS2 predisposes to both malignant and nonmalignant lymphoproliferation,[30-32] our

Table 2 Biochemical and immunological laboratory workup of the APDS2 and SHORT patient

Test	Age	
	18 months	36 months
Biochemistry		
• Glucose profile		
Glucose mg/dL		81 [60-100]
Insulin kU/mL		2,0 [<15]
HbA1c %		5,0 [<5,7]
• Lipid profile		
Cholesterol mg/dL		123 [110-230]
HDL mg/dL		51 [>5]
LDL mg/dL		64 [<135]
Triglicerides mg/dL		39 [31-96]
Immunology		
• Immunoglobulins		
IgG mg/dL	403 [520-1360]	
IgA mg/dL	25 [45-135]	
IgM mg/dL	27 [46-190]	
• IgE profile		
Total IgE kU/L	629 [<28,7]	
α-lactoalbumin kU/L	1,8 [<0,35]	
β-lactoglobulin kU/L	9,6 [<0,35]	
caseine kU/L	0,36 [<0,35]	
• Lymphocyte immunophenotype		
lymphocytes CD45+/SSC low %, cc/mcL		On IVIg 863 [540-1420]
B CD19+ %, cc/mcL		53 [52-220]
T CD3+ %, cc/mcL		**15** [40-200]
NK CD3-CD45+CD16+CD56+ %, cc/mcL		39,0, 3860 [29-46, 1400-5500]
Transitional B CD19+CD38+IgM++ %, cc/mcL		11,0, 425 [8-39, 180-1300]
Mature naïve B CD19+CD27-IgD+ %, cc/mcL		67,0, 2618 [52-92, 850-4300]
Non-switched memory B (MZL) CD19+CD27+IgD+ %, cc/mcL		16,0, **645** [2-25, 61-510]
Switched memory B CD19+CD27+IgD- %, cc/mcL		5,3, 23 [3,1-12,3, 20-200]
Immature B CD19+CD21lo %, cc/mcL		76,2, 324 [54,0-88,4, 280-1330]
Activated B CD19+CD38loCD21lo %, cc/mcL		8,8, 37 [2,7-19,8, 20-180]
Plasmablasts CD19+CD38++IgM- %, cc/mcL		11,6, 49 [4,7-21,2, 20-220]
T helper CD3+CD4+ %, cc/mcL		7,4, 31 [4,1-24,4, 20-230]
T suppressor/cytotoxic CD3+CD8+ %, cc/mcL		**7,4**, 31 [1,7-5,4, 10-60]
CD4+/CD8+		**0,5**, **2** [0,6-4,0, 10-50]
Recent thymic emigrants CD3+CD4+CD45RA+CD31+ %, cc/mcL		41,0, 1596 [25-66, 500-2700]
Naïve T helper CD3+CD4+CD45RA+CD27+ %, cc/mcL		22,0, 861 (9-49, 200-1800]
Central memory T helper CD3+CD4+CD45RA-CD27+ %, cc/mcL		1,85
Effector memory T helper CD3+CD4+CD45RA-CD27- %, cc/mcL		81,1, 1294 [37-100, 190-2600]
Terminally differentiated memory T helper CD3+CD4+CD45RA+CD27- %, cc/mcL		**95,3**, 1520 (52-92, 300-2300] **3,3**, **52** [15-56, 160-660]
Follicular CXCR5+ T helper CD3+CD4+CD45RO+CD185+ %, cc/mcL		0,8, 13 [0,3-9, 3-89]
Regulatory T helper CD3+CD4+CD25++CD127- %, cc/mcL		0,6, 10 [0-1,2, 0,0-16]
Naïve T suppressor/cytotoxic CD3+CD8+CD27+CD197+ %, cc/mcL		9,1, **7** [6-72, 13-170]
Central memory T suppressor/cytotoxic CD3+CD8+CD45RA-CD27+CD197+ %, cc/mcL		**1,7**, **27** [3-17, 39-150] 59,3, 511 [19-100%, 53-1100]
Effector memory T suppressor/cytotoxic CD3+CD8+CD45RA-CD27-CD197- %, cc/mcL		1,0, 9 [1-9, 4-64] **3,3**, 29 [10-55, 24-590]
Terminally differentiated T suppressor/cytotoxic CD3+CD8+CD45RA+CD27-CD197- %, cc/mcL		23,9, 206 [6-83, 25-530]
• Lymphocyte proliferation assays		
Anti-CD3, PHA, Pansorbin Cells stimulation indexes		normal
Microbiology		
• Nasopharyngeal aspirate culture	*H. influenzae*	*H. influenzae*, *S. pneumoniae*, *P. aeruginosa*, *S. marcescens*
• Nasopharyngeal aspirate viral test	*Rhinovirus*	*Rhinovirus*

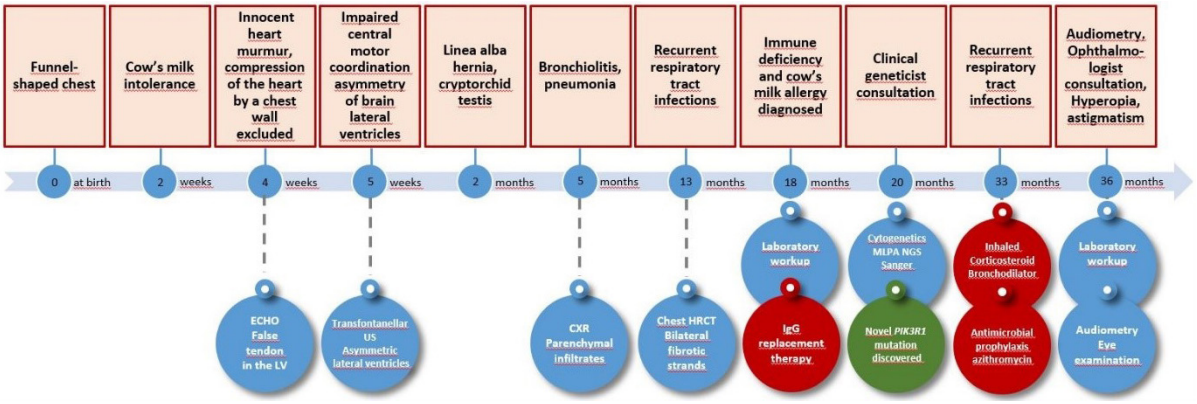

Figure 2 Timeline showing relevant data from the episode of patient's care.

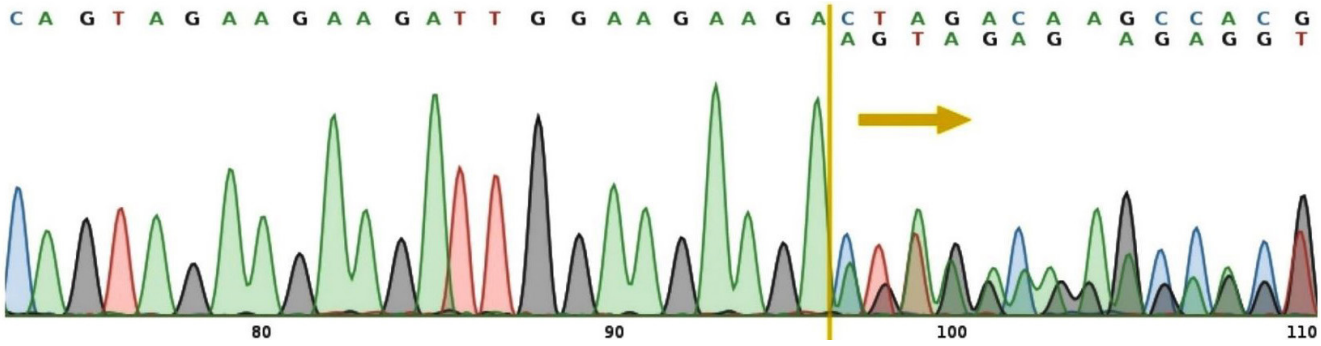

Figure 3 The novel mutation in the *PIK3R1* gene discovered. Sanger Sequencing results of *PIK3R1* DNA sequence (NM_181523.2). The yellow arrow indicates where the mutation c.1644_1648delCTTGA (p.Asp548Glufs*6) occurs in the DNA sequence and generates a frameshift that interferes with further reading of the sequence.

patient requires regular monitoring of immunological parameters. It needs to be highlighted that the natural history of patients with PIK3R1-related conditions is markedly heterogeneous, ranging from an early-life lymphoproliferative disease and severe infections[28] to asymptomatic patients[18] receiving live vaccines with no AEFI.[28] Further therapeutic options, such as antibiotic prophylaxis, mTOR inhibition-targeted therapies with rapamycin and selective PI3Kδ inhibitor leniolisib, as well as hematopoietic stem cell transplantation (HSCT), require future considerations.[33] However, it is worth noting that peripheral blood lymph cell flow cytometric immunophenotyping does not show significant developmental abnormalities of the B and T cell compartments. Currently, at the age of 3 years, the patient does not present severe infections or signs and symptoms of autoimmune disorders, and lymphoproliferative disease. The symptomatology of SHORT syndrome is mild, with normal psychomotor and speech development, satisfactory linear growth velocity, and normal teething, and absent anterior chamber ocular abnormalities, facial dysmorphism, lipodystrophy, and metabolic disorders. The newly diagnosed abnormality in the patient is hyperopia (+1,25 D bilaterally) with astigmatism, which was already observed in a pediatric case of the SHORT syndrome.[2] Whereas some phenotypic features of the SHORT syndrome, such as dyslipidemia and glucose intolerance, may develop in the second decade of life, and screening procedures for early detection of these complications need to be undertaken. Concurrently, in this report, we add new phenotypic features in the form of CCWD, pectus excavatum, a stellate pattern of the iris, and dysplastic nails to the clinical set of symptoms of the SHORT syndrome. Likewise, hyperimmunoglobulinemia E and hypersensitivity to cow's milk allergens have not been identified either in patients affected with the SHORT syndrome or APDS2. As atopy may reflect an immune dysregulation being a part of mTOR-opathy, in our patient, it might be considered as an overlapping pathophysiology and a clinical link between APDS2 and SHORT syndrome. The presence of the same variant in the maternal DNA may be due to its incomplete penetrance, but other protective roles of yet undefined genetic or environmental factors might also be considered.

A molecular pathway related to a novel c.1644-1648del (p.Asp548Glufs*6) mutation in the *PIK3R1* gene is associated with concomitant APDS2 mTOR-opathy and SHORT syndrome, and an expanding combined clinical phenotype with CCWD and IgE-mediated hypersensitivity to cow's milk. It may therefore be hypothesized that these unique phenotypic features imply a multiplicity of biological roles of PIK3δ in both immune homeostasis as well as in extraimmune development and physiology of the central nervous system, growth potential, hormonal balance, and organ development. Nevertheless, due to the novelty of the c.1644-1648 frameshift mutation in *PIK3R1*, a multidisciplinary approach and regular monitoring of the patient will provide data on the clinical pathogenicity of the mutation. Determination of the inhibitory or activating role of this novel mutation by functional validation studies might

shed light on the genotype-phenotype correlations in the patient and his mother.

An individualized approach and multidisciplinary care of specialists in pediatric laryngology, cardiology, ophthalmology, endocrinology, hematooncology, psychology, and medical genetics is being currently accomplished. Further considerations and decisions regarding therapeutic options, such as antibiotic prophylaxis, mTOR inhibition-targeted therapies with rapamycin and selective PI3Kδ inhibitor, leniolisib, as well as HSCT, are required. Further APDS2 and SHORT syndromes definition and delineation, with their phenotype-genotype interrelated correlations, are required. Further clinical observation on developing infectious, autoimmune, and lymphoproliferative disorders as well as immunological evaluation of the patient's mother is being carried out. From the patient's perspective, it could facilitate an individualized approach and implementation of multidisciplinary care of specialists in pediatric pulmonology, laryngology, cardiology, ophthalmology, endocrinology, hematooncology, psychology, and medical genetics under the clinical immunologist's supervision.

Consent

Written informed consent was obtained from the parents of the patient for the publication of any potentially identifiable images or data included in this article.

Author Contributions

AS-P was responsible for the conception and design of the study, collection, and interpretation of clinical data and drafted the manuscript. KB-S was involved in the collection of clinical data and participated in drafting the manuscript. ES helped in collecting clinical data and was involved in drafting the manuscript. MP performed Sanger sequencing and was responsible for the interpretation of genetic testing. MB-S was responsible for the interpretation of genetic testing and participated in drafting the manuscript.

Conflict of Interest Statement

The submitted work was carried out in the presence of any personal, professional, or financial relationships that could potentially be construed as a conflict of interest.

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