

Activating PI3K δ mutations in a cohort of 669 patients with primary immunodeficiency

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Accepted for publication 17 September 2015
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Introduction

Genetic defects of the immune system impair host defence and lead to an increased susceptibility to recurrent, severe or unusual infections. There are more than 200 Mendelian traits described to cause immunodeficiency [1]. Defects affecting the humoral branch of the adaptive immune system are either caused by intrinsic dysfunction of B cells and their capacity to produce specific neutralizing antibodies or by insufficient T cell help in the germinal centres of lymph

Summary

The gene *PIK3CD* codes for the catalytic subunit of phosphoinositide 3-kinase δ (PI3K δ), and is expressed solely in leucocytes. Activating mutations of *PIK3CD* have been described to cause an autosomal dominant immunodeficiency that shares clinical features with common variable immunodeficiency (CVID). We screened a cohort of 669 molecularly undefined primary immunodeficiency patients for five reported mutations (four gain-of-function mutations in *PIK3CD* and a loss of function mutation in *PIK3R1*) using pyrosequencing. *PIK3CD* mutations were identified in three siblings diagnosed with CVID and two sporadic cases with a combined immunodeficiency (CID). The *PIK3R1* mutation was not identified in the cohort. Our patients with activated PI3K δ syndrome (APDS) showed a range of clinical and immunological findings, even within a single family, but shared a reduction in naive T cells. *PIK3CD* gain of function mutations are more likely to occur in patients with defective B and T cell responses and should be screened for in CVID and CID, but are less likely in patients with a pure B cell/hypogammaglobulinaemia phenotype.

Keywords: B cells, common variable immunodeficiency, hypogammaglobulinaemia, PI3K δ , primary immunodeficiency

nodes and tonsils. In humans, this dysfunction may manifest as a syndrome known as common variable immunodeficiency (CVID), characterized by hypogammaglobulinaemia and recurrent infections, predominantly of the respiratory tract and the gut [2].

Activating mutations in p110 δ , the catalytic subunit of phosphoinositide 3-kinase δ (PI3K δ) have been demonstrated to cause activated PI3K δ syndrome (APDS, MIM615513), with an autosomal dominant pattern of inheritance. PI3K δ belongs to the class IA phospho-

inositide-3-kinases (PI3Ks) and is expressed exclusively in leucocytes [3,4]. The protein is composed of a regulatory subunit, p85, and the catalytic subunit p110 δ which phosphorylates PI(4,5)P₂ to PI(3,4,5)P₃ (PIP₃) at the plasma membrane of lymphocytes upon receptor activation leading to downstream Akt/mTOR signalling with a consequent impact on T (and B) cell activation and fate [5].

In APDS, increased Akt/mTOR signalling leads to an immune dysregulation and an immunodeficiency, characterized by respiratory infections, bronchiectasis and autoimmune cytopenias [6,7]. Loss of function mutations in phosphatidylinositol 3-kinase regulatory (*PIK3R1*) encoding the p85 α regulatory subunit also result in hyperactivation of PI3K signalling with the same phenotype resulting from *PIK3CD* gain of function mutations, namely immunodeficiency, lymphoproliferation, poor antibody responses and expansion of senescent CD8⁺ T cells [8,9]. Immunological findings described previously in APDS include B cell lymphopenia with relatively increased transitional B cell numbers and reduced immunoglobulin (Ig)G, but elevated IgM levels in serum [6,7], features that are shared partially with CVID [10]. The differential diagnosis of APDS also extends to combined immunodeficiency (CID) or 'atypical' severe combined immunodeficiency (SCID) (defined as immunodeficiency due to mutations in SCID-causing genes in patients with a presentation different from typical SCID and Omenn syndrome and T cell levels above 500 cells/ μ l [11,12]). CID patients present above the age of 1 year with clinical features that can include bronchiectasis, autoimmune cytopenia, recurrent and prolonged viral infection, lymphopenia, restricted antibody response and Epstein–Barr virus (EBV)-associated lymphoproliferation [13]. Clinical overlap with other primary antibody deficiencies, including X-linked agammaglobulinaemia and hyper-IgM syndrome, has been noted [14].

PI3K has been implicated previously in haematological malignancies, including B cell lymphomas [15–17]. Correspondingly, patients with activating mutations of PI3K δ have been described to exhibit benign and malignant lymphoproliferative disease, often in association with EBV viraemia [7]. Among eight APDS patients reported by Kracker and co-authors, two developed B cell lymphoma [18]. Crank and co-workers identified another pathogenic activating mutation in the p110 δ subunit in patients with hyper-IgM syndrome who also developed lymphoproliferative syndromes, although not in association with EBV in their cohort [19].

Among the genetically defined immunodeficiencies which are not lethal in infancy, APDS is of particular interest because commercially available inhibitors of PI3K may represent a specific therapeutic option [15,20]. Previous reports did not explore the incidence of *PIK3CD* and *PIK3R1* mutations in patients with undefined hypogammaglobulinaemia. We therefore probed for the four published *PIK3CD* gain-of-function mutations and one splice site

mutation in *PIK3R1* in a large European cohort of predominantly CVID patients and patients with other primary antibody deficiencies.

Methods

Patients

A total of 669 immunodeficiency patients, mainly from continental Europe, were included in this screen: 610 patients diagnosed with CVID, 10 patients with an autoimmune lymphoproliferative syndrome (ALPS) phenotype but no identifiable defect in the Fas-apoptotic pathway, 10 patients with a diagnosis of hyper-IgM syndrome, 10 patients with a specific antibody deficiency, six patients with a combined immunodeficiency (CID) phenotype [11], five patients with selective IgA deficiency, two patients with agammaglobulinaemia and 16 patients with other minor antibody deficiencies.

The gender distribution of the cohort was almost equal, 49% male and 51% female. Almost 14% were diagnosed at age ≤ 10 years, 20% at age > 10 – ≤ 20 years, 41% at age > 20 – ≤ 40 years, 22% at age > 40 – ≤ 60 years, and 3% at age > 60 years. Patients in this multi-centre cohort were diagnosed according to the criteria of the European Society for Immunodeficiency (ESID) and the Pan-American Group for Immunodeficiency (PAGID) (available at www.esid.org). A total of 416 patients were recruited at the University Medical Centre Freiburg in Germany, 112 at Oslo University Hospital in Norway and 141 at the Royal Free Hospital in London, UK.

All individuals donated samples following written informed consent. This study was approved by the ethics review board of the Albert Ludwig University Freiburg, Germany (protocols 239/99_BG, 251/13_KW and 282/11_SE version 140023), the research ethics committee of the Royal Free Hospital and Medical School, London, UK (protocols #04/Q0501/119_AM03 for affected individuals, #07/H0720/182 for family members and #08/H0720/46 for healthy controls) and the regional committees for medical and health research ethics, Norway (reference number REK 2.2007.201).

Target mutations

Four gain-of-function mutations in *PIK3CD* encoding the catalytic p110 δ subunit were targeted for screening, namely: C \rightarrow A at cDNA position c.1002 resulting in the amino acid substitution N334K in the C2 domain; G \rightarrow A at position c.1573 with consequent E525K substitution in the helical domain; G \rightarrow A at position c.3061 resulting in an E1021K substitution in the C-lobe of the kinase domain [[6],7]; and T \rightarrow C at position c.1246, resulting in the amino acid substitution C416R in the C2 domain [19]. The study population was screened further for a splice site mutation

Table 1. Overview of screen for reported PI3K δ and p85 α mutations in 669 immunodeficiency patients from continental Europe

Diagnosis*	Number of patients	Screened mutations				
		PIK3CD				PIK3R1
		E1021K	E525K	N334K	C416R [†]	c.1425 + 1
CVID	610	3	0	0	0	0
ALPS-phenotype	10	0	0	0	0	0
HIGM	10	0	0	0	0	0
SPAD	10	0	0	0	0	0
sIgAD	5	0	0	0	0	0
Agammaglobulinaemia	2	0	0	0	0	0
CID-phenotype	6	2	0	0	0	0
Others	16	0	0	0	0	0
Total	669	5	0	0	0	0

*ALPS = autoimmune lymphoproliferative syndrome. [†]Mutation screen was performed in 627 samples. CVID = common variable immunodeficiency; CID = combined immunodeficiency; HIGM = hyper-immunoglobulin (Ig)M syndrome; SPAD = specific antibody deficiency; sIgAD = selective IgA deficiency; others = other minor forms of hypogammaglobulinaemia.

in *PIK3R1* corresponding to c.1425 + 1 that results in skipping of exon 11 of the gene with in-frame deletion of the nucleotides encoding the amino acid residues 434–475 of p85 α , which constitute part of the inter-SH2 domain common to *PIK3R1* isoforms [8,9]. No search for new possible mutations was undertaken in this study.

Pyrosequencing

Pyrosequencing technology was employed to detect the five mutations under investigation. Assays were designed using the PyroMark Assay software version 2.0.1.15 (Qiagen, Hilden, Germany). All primers were obtained from Aparas Biosciences GmbH (Denzingen, Germany). Target genomic DNA fragments were amplified by polymerase chain reaction (PCR) using the primers detailed in Supporting information, Table S1; 5 pmol each of the forward and reverse primers, one being biotinylated, were employed to amplify the fragments of interest in a final reaction volume of 12 μ l. PCR products were rendered single-stranded according to an established protocol [21]. Three pmol of the sequencing primers provided in Supporting information, Table S1 were used in each case to carry out pyrosequencing of the amplified fragments on the PyroMark Q96 MD apparatus (Qiagen). Samples were amplified and sequenced in 96-well plates.

Immunophenotyping

Measurement of immunoglobulin levels and lymphocyte immunophenotyping was performed at the Centre of Chronic Immunodeficiency, University Medical Centre Freiburg, Germany, and the Institute of Immunity and Transplantation at the University College London. Paediatric immunological investigations on the UK patients were performed at Great Ormond Street Hospital, London.

Results

A total of 669 immunodeficiency patients were screened for the reported gain-of-function mutations in *PIK3CD*, N334K, E525K, E1021K and C416R, and a splice site mutation in *PIK3R1*. We identified five patients with the heterozygous E1021K mutation in *PIK3CD*: a family of three siblings (family A) and two sporadic cases. None of the other mutations were observed. Results of the mutation screen are presented in Table 1. Despite being first-degree relatives, with an identical mutation, the three siblings in family A, referred to further as siblings 1, 2 and 3, displayed diverse clinical and immunological features reminiscent of CVID, as detailed below and in Table 2. The two sporadic cases with the E1021K mutation were diagnosed with CID, distinguished from the three siblings by their elevated IgM levels, and are referred to below as sporadic patients (SP) 1 and 2.

Case histories

CVID siblings (family A). The eldest sibling, a male now aged 26 (sibling 1), was unwell from the first year of life with frequent respiratory tract infections. He suffered epiglottitis at 18 months of age and was ventilated for almost 2 weeks; at that time he was found to be IgA-deficient. He continued to experience recurrent sinus, ear and respiratory infections and was diagnosed with mild bronchiectasis at the age of 2 years. His immune system was not fully re-evaluated until his sister (sibling 2 – see below) was diagnosed with antibody deficiency; at that time sibling 1 was aged 5 years. He was found to be borderline IgG-deficient (4.4 g/l) with normal IgM (see Table 2). He was commenced on intravenous immunoglobulin (IVIG) replacement, which he discontinued at age 16 years. He has been prescribed co-trimoxazole prophylaxis, but currently does not take it. He still suffers from occasional respiratory

Table 2. Clinical and immunological features of activated PI3K δ syndrome (APDS) patients

	Sibling 1	Sibling 2	Sibling 3	Sporadic patient 1	Sporadic patient 2
Current Age (years)	26	21	20	15	9
Sex	Male	Female	Female	Male	Male
Age at diagnosis of antibody deficiency (years)	5	1	1	9	3
Chest infections	Yes	Yes	Yes	Yes	Yes
Bronchiectasis	Yes (minimal)	Yes	No	Yes	Yes
CVID enteropathy	No	Yes	No	No	Yes
Chronic rhinosinusitis	No	Yes	Yes	No	No
Asthma	No	Yes	No	No	No
Lymphadenopathy	Yes	Yes	Yes	Yes	Yes
Splenomegaly	Yes	No	Yes	Yes	Yes
EBV viraemia	Yes	No	No	No	No
Other clinical features	Nil	Eczema	Exocrine pancreatic dysfunction	Thyroiditis, AIHA, ITP	Mucocutaneous candidiasis,systemic CMV infection,adenovirus viraemia
On Ig replacement	No	Yes	Yes	Yes	Yes
IgG at initial diagnosis prior to antibody replacement (g/l) (NR)	4.4 (4.9–16.1)	0.54 (3.1–13.8)	0.95 (3.1–13.8)	1.78 (4.9–16.1)	1.42 (4.2–16.1)
IgM at initial diagnosis prior to antibody replacement (g/l) (NR)	0.96 (0.5–2.0)	0.95 (0.5–2.0)	1.26 (0.5–2.0)	4.86 (0.5–2.0)	0.52 (0.5–2.0)
Total lymphocyte count ($\times 10^6/l$) (NR)	1345 (1000–2800)	1005 (1000–2800)	946 (1000–2800)	584 (1100–5900)	1220 (1100–5900)
CD3 ⁺ T cells ($\times 10^6/l$) (NR)	1035 (700–2100)	800 (700–2100)	718 (700–2100)	314 (700–4200)	1076 (700–4200)
CD4 ⁺ T cells ($\times 10^6/l$) (NR)	490 (300–1400)	403 (300–1400)	322 (300–1400)	180 (300–2000)	226 (300–2000)
CD8 ⁺ T cells ($\times 10^6/l$) (NR)	522 (200–900)	370 (200–900)	368 (200–900)	100 (300–1800)	839 (300–1800)
CD4/CD8 ratio	0.94	1.09	0.88	1.72	0.27
Naïve T cell (CD4 ⁺ CD45RA ⁺) % (NR)	19 (33–66)	24 (33–66)	14 (33–66)	14.3 (46–77)	7.4 (46–77)
Memory T cell (CD4 ⁺ CD45RO ⁺) % (NR)	84 (30–58)	79 (30–58)	88 (30–58)	44 (13–30)	86.5 (13–30)
B cell count ($\times 10^6/l$) (NR)	225 (100–500)	41 (100–500)	138 (100–500)	28 (100–500)	62 (100–500)
Switched memory B cell % (IgD ⁻ CD27 ⁺)	36.1	1.7	3.5	27.4	NA
Transitional B cell % (CD24 ⁺ CD38 ⁺)	0.1	90.9	67.3	0.07	NA

NR = normal range; Ig = immunoglobulin; CVID = common variable immunodeficiency; AIHA = autoimmune haemolytic anaemia; IT = idiopathic thrombocytopenic purpura; EBV = Epstein–Barr virus; CMV = cytomegalovirus.

infections and has minimal bronchiectasis (Fig. 1). He also has splenomegaly (15 cm) and a persistent low-level EBV viraemia.

The second sibling (sibling 2), a 21-year-old female, has suffered since infancy from recurrent lower respiratory infections, chronic rhinosinusitis and colitis. Identified initially to be antibody-deficient with very low levels of IgG (0.54 g/l), but normal IgM (Table 2), she was diagnosed subsequently with CVID. She remains on IVIG infusions and prophylactic antibiotics and so far has moderate bronchiectasis (Fig. 1). Recent respiratory pathogens identified include *Streptococcus pneumoniae* and *Haemophilus influenzae*; she has previously required eradication therapy for *Pseudomonas aeruginosa*. She also has mild eczema. A

particular issue for sibling 2 has been bowel inflammation with diarrhoea. Biopsies of the stomach and colon have demonstrated a polyclonal lymphocytic infiltrate within the lamina propria, associated with meshworks of CD21⁺ follicular dendritic cells. A lymphoid nodule has been discovered within the stomach, while in the colon, among a background inflammatory change and ulceration, there was possible epithelial infiltration by IgM⁺ lymphocytes. Although these infiltrating cells were not monoclonal, on one occasion a VH4 clone was discovered in peripheral blood on immunoglobulin heavy chain analysis. There is no other evidence of lymphoproliferative disease and PCR for EBV is negative; the spleen is at the upper limit of normal (13 cm). In 2014, sibling 2 had a norovirus gastrointestinal



Fig. 1. Spectrum of bronchial disease in family A siblings. Chest computed tomography (CT) scans of siblings 1, 2 and 3 (a,b,c) showing minimal bronchiectasis in sibling 1 (a), moderate bronchiectasis in sibling 2 (b) and unaffected lung tissue in sibling 3 (c).

infection, which persisted for several weeks but eventually cleared in stool spontaneously (defined as consistently negative viral PCRs for several months); this correlated with a reduction in clinical symptoms, but some diarrhoea still persisted. After clearance of the norovirus infection she was commenced on a tapering dose of prednisolone and maintenance mesalazine, with good clinical response.

The youngest female sibling (sibling 3), now aged 20 years, was diagnosed with hypogammaglobulinaemia at the age of 1 year (IgG 0.95 g/l), again with normal IgM (Table 2). She was commenced on immunoglobulin replacement at that time. She had a significant varicella zoster infection at the age of 2.5 years with a large number of lesions, but did not require hospitalization. She had an episode of Henoch–Schönlein purpura at the age of 11 and was also diagnosed in the same year with molluscum contagiosum. This particularly affected the buttocks and hips and lasted for approximately 1 year. However, she has suffered only occasional respiratory tract infections and has a normal computed tomography (CT) scan of the chest (Fig. 1). She continues to suffer from pharyngitis intermittently and is symptomatic from a perennial rhinitis. She also has splenomegaly (15.3 cm) and mild thrombocytopenia (current platelet count $124 \times 10^9/l$), but EBV viraemia has not been demonstrated. Sibling 3's clinical picture is dominated currently by acquired exocrine pancreatic dysfunction with malabsorption, which was diagnosed at approximately 6 years of age. She passes multiple greasy stools per day despite supplementation with pancrelipase, and was found recently to be deficient in vitamin E [4.6 $\mu\text{mol/l}$ (NR 11.6–46.4 $\mu\text{mol/l}$)]. She does not have diabetes mellitus and has no evidence of inflammation of the gastrointestinal tract on multiple endoscopies.

This clinical heterogeneity correlates with differences in immunological findings. In particular, the proportion of CD27⁺ IgD class-switched memory B cells differs significantly between the siblings, with the lowest levels seen in sibling 2, who has the highest burden of sinopulmonary infection, bronchiectasis and inflammatory bowel disease (Fig. 1 and Table 2). Transitional B cell percentages demonstrate a reciprocal pattern, with the highest percentage

reported in sibling 2. The male sibling (sibling 1) has a relatively normal B cell profile. All three siblings demonstrate reduction in the proportion of CD4⁺ cells expressing CD45RA⁺ (sibling 1, 19%, sibling 2, 24%, sibling 3, 14% versus a normal reference range of 33–66%).

Importantly, both parents of the three siblings are healthy and tested negative in blood for the E1021K mutation in p110 δ .

Sporadic CID patients. SP1 is the only child of non-consanguineous German parents, now aged 15 years. At 6 years of age he developed his first bacterial pneumonia. A second pneumonia, in combination with purulent sinusitis and otitis media, manifested at 6 years of life. One month later, a first episode of severe autoimmune haemolytic anaemia (AIHA) occurred (nadir Hb 4.9 g/dl), which at this time responded to short-term steroid treatment. Aside from some mild upper respiratory infections his further clinical course was stable until 8 years of life, when he was admitted with an abscess of a cervical lymph node, which required surgical intervention (cultures positive for *S. pneumoniae*) and bilateral bacterial pneumonia. Splenomegaly was noted for the first time and progressed up to his umbilicus within a few months (Fig. 3). Shortly after, he suffered another episode of AIHA which has relapsed multiple times (often in response to respiratory viral infection) ever since.

Severely reduced IgG levels (1.78 g/l) and elevated polyclonal IgM (4.86 g/l) were recognized at 9 years of age and IgG replacement therapy was initiated. A CT scan at this time identified significant bronchiectasis and mediastinal lymphadenopathy, but no interstitial lung disease (Fig. 2). At age 10 years, idiopathic thrombocytopenic purpura (ITP) and neutropenia occurred and mycophenolate mofetil (MMF) was introduced as a steroid sparing agent. Chronic lymphopenia (below $1000 \times 10^6/l$) was documented in all available blood counts since age 9 and was present before the introduction of any immunosuppressive treatment.

Lymphocyte subset analysis over time demonstrated progressive B and T cell lymphopenia including loss of naive T cells but normal T cell proliferation to mitogens.



Fig. 2. Lung disease in sporadic patient 1 (SP1). Axial chest computed tomography (CT) scans of SP1 demonstrating pronounced bronchiectasis, mediastinal lymphadenopathy, peribronchial inflammation but no interstitial disease.

Total lymphocyte, T and B cell counts dropped from 1650, 1472 and $93 \times 10^6/l$ in 2008 to 584, 314 and $28 \times 10^6/l$ in 2013, respectively (Table 2). Among his reduced total B cells the fractions of plasmablasts and CD21-low B cells were relatively increased. At 11 years, attacks of AIHA and ITP worsened and were controlled only transiently by various immunosuppressive regimens, including steroid pulses, rituximab and mTOR pathway inhibition with sirolimus. Both ITP and AIHA improved eventually after splenectomy at 12 years of age. Prior to this splenectomy he also developed maturation arrest of granulopoiesis, which was treated with granulocyte-colony stimulating factor (G-CSF). However, this condition also improved post-splenectomy. The previous use of sirolimus for 6 months did not result in a reduction of his spleen size or improvement of cellular phenotype and was discontinued when the patient developed hypertension and neutropenia. Histological sections of resected lymph nodes, spleen and liver are provided in Supporting information, Fig. S1.

SP2 is the first child of non-consanguineous Polish parents. He is now aged 9 years, with a younger healthy sibling. He had severe enteropathy with follicular hyperplasia presenting as chronic diarrhoea from the age of 2 months. He suffered from severe recurrent lower respiratory tract infections from the age of 7 months (Fig. 4). Onset of lym-

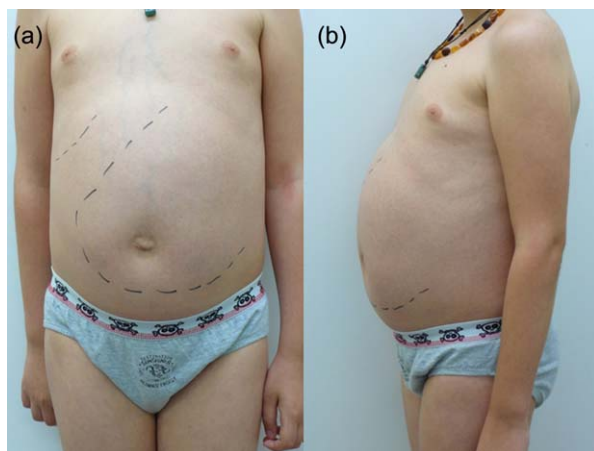


Fig. 3. Massive splenomegaly in sporadic patient 1 (SP1). Ventral (a) and lateral (b) images of abdomen in SP1 showing massive splenomegaly.

phoproliferation was from age 2 years onwards, when he first presented with generalized lymphadenopathy and marked splenomegaly. He was placed on subcutaneous immunoglobulin (SCIG) replacement therapy after hypogammaglobulinaemia which was recognized at age 3 and the frequency of his previous recurrent bacterial pneumonias improved. Other infectious complications included systemic cytomegalovirus (CMV) infection at the age of 4 years, mucocutaneous candidiasis at 5 years and chronic adenovirus viraemia detected at 8 years of age.

Immunologically, he had profound hypogammaglobulinaemia (IgG 1.42 g/l and IgA undetectable) at age 3 years and an initially normal IgM (0.52 g/l) level that increased to 3.34 g/l by 8 years of age. His blood counts showed mild thrombocytopenia $86 \times 10^9/l$, a reduction of total B cell, CD4⁺ and naive T cell counts, but normal T cell proliferation to mitogens.

Both SP1 and SP2 were classified clinically as cases of CID based on the presence of severe immune dysregulation, chronic systemic infections despite immunoglobulin substitution, T cell lymphopenia and progressive loss of naive T cells.

Discussion

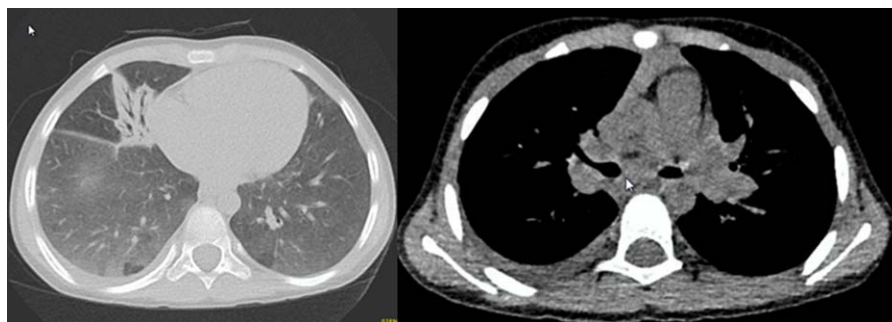
We identified five patients with the heterozygous gain of function mutation E1021K in the gene *PIK3CD* encoding PI3K δ in a large multi-centre European cohort of 669 patients diagnosed with antibody deficiencies. Three siblings from the same family had been diagnosed previously with CVID and two sporadic cases were diagnosed previously as CID.

Our findings contrast with the higher mutation frequency established by Lucas *et al.* [7] and Angulo *et al.* [6]. The latter group had excluded a founder effect in their UK/Ireland cohort [6]. We therefore believe that the low frequency we have observed is explained by the specific cohort we have studied, consisting mainly of antibody-deficient patients.

Indeed, our report is the first to document APDS among patients with CVID and highlights the need to consider APDS in the differential diagnosis of this condition.

Our findings underline the variability of the APDS phenotype, even within one family. Importantly, APDS may

Fig. 4. Lung disease in sporadic patient 2 (SP2). Axial chest computed tomography (CT) scans of SP2 demonstrating mucus impaction of the left middle lobe, moderate bronchiectasis, interstitial disease and enlargement of mediastinal lymph nodes.



present without elevation of IgM and be diagnosed as CVID, emphasizing the importance of screening patient cohorts with this diagnosis. The phenotype in this family corresponded to features associated with CVID such as persistent hypogammaglobulinaemia, respiratory infection, bronchiectasis, splenomegaly and low-level EBV viraemia. Importantly, even the sibling with relatively few infections (sibling 1) has potentially premalignant risk factors (EBV viraemia and splenomegaly). Similarly, his more clinically affected sister (sibling 2) shows epithelial lymphocytic infiltration in the colonic mucosa.

The two sporadic patients carrying a mutant *PIK3CD* allele were diagnosed previously with CID, involving both the B and T cell compartment. Bronchiectasis was pronounced in both cases and lymphoproliferation was a common feature. Both had low numbers of mature and naive T cells together with low B cell counts, low IgG levels and elevated IgM. Their clinical and immunological phenotype greatly resembled previously reported APDS patients [6,7] with respiratory infections, bronchiectasis humoral immunodeficiency and progressive B cell lymphopenia with normal or reduced T cells, reduction in class-switched memory B cells, increased IgM and reduced IgG2 levels. This profile of combined immunodeficiency, lymphoproliferation and inflammation is shared with patients carrying the splice site mutation in *PIK3R1* with subsequent hyperactivation of PI3K due to impaired association with p110 δ [8,9], although we did not identify any patients with this mutation in our cohort.

APDS shares clinical parallels with other antibody deficiencies that exhibit a combined immunodeficiency phenotype such as hyper-IgM syndrome [22], as demonstrated by the identification of *PIK3CD* mutations in patients diagnosed with this condition [19]. The cohort investigated here included only a limited number of hyper-IgM syndrome patients, and none carried any of the five reported APDS mutations. Autoimmune phenomena, enteropathies and blood dyscrasias feature strongly in lipopolysaccharide-responsive and beige-like anchor protein (*LRBA*) deficiency, another condition with a profile of combined immunodeficiency and autoimmunity [23,24], while massive EBV-driven B cell lymphoproliferation and possibly EBV-associated lymphoma is characteristic of deficiencies of

interleukin 2-inducible T cell kinase (*ITK*) [25–27], CD27 [28,29] and X-linked lymphoproliferative disease caused by mutations in *SH2D1A* [30].

The triad of severe immunodeficiency, microthrombocytopenia and eczema with often high IgE levels sets Wiskott–Aldrich syndrome apart from the above, but a clinical overlap with APDS is worth noting [31,32]. The recently described autosomal dominant syndrome of cytotoxic T lymphocyte-associated protein 4 (*CTLA4*) deficiency shares a clinical picture of hypogammaglobulinaemia, lymphoproliferation, progressive loss of B cells, recurrent infections and prominent autoimmune features [33,34] with APDS, although patients with *CTLA4* deficiency tend to have a later onset of disease [33].

Taking note of the phenotypical heterogeneity of APDS, the following features are observed most frequently and should alert the clinician to a possible diagnosis: sporadic or autosomal dominant transmission, bacterial and viral infections, lymphadenopathies, normal or often increased serum IgM levels, increased frequency of transitional B cells, decreased numbers of naive CD4 and CD8 T cells and increased numbers of CD8 effector/memory T cells.

In summary, we have demonstrated that APDS, although rare within our cohort of primary immunodeficiency patients, has diverse clinical and immunological characteristics (even within a single family), making it hard to differentiate from other primary immunodeficiencies. Of particular concern is the risk of malignancy in this syndrome, and thus screening for *PIK3CD* mutations and consideration of targeted treatment is warranted in patients with a current diagnosis of CVID or CID.

We conclude that activating mutations in *PIK3CD* may cause immunodeficiency in patients with a complex phenotype combining defective B and T cell responses, but are less likely to be discovered in patients where only the humoral arm of the immune response is affected and naive T cell numbers are normal. APDS should be considered in the differential diagnosis of CVID and CID.

Acknowledgements

This study was supported by the Excellence Initiative of the German Research Foundation (GSC-4, Spemann Graduate

School) and by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung) grants 01EO0803 and 01GM1111B.

Disclosure

The authors declare that they have no disclosures.

Author contributions

M. E., D. S., J. H., Z. E. and A. D. carried out the experiments in the laboratories of A. N. and B. G.; D. M. L. and C. S. provided clinical data on patients. A. S. G. performed histological examinations. D. M. L., C. S., B. F., U. S., S. B. and B. G. contributed patient samples. J. W., S. F. J. and D. S. prepared DNA aliquots. M. E., D. M. L. and C. S. drafted the manuscript with contributions from D. S.; B. G. conceived the study. S. F. J., B. F., U. S., A. N., S. B. and B. G. reviewed the manuscript and M. E. prepared the manuscript for submission. We thank Sarita Workman for her contribution.

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Supporting information

Additional Supporting information may be found in the online version of this article at the publisher's web-site:

Table S1. Primer sets for pyrosequencing of *PIK3CD* and *PIK3R1* mutations.

Fig. S1. Representative tissue sections of lymph node (a), spleen (b–f) and liver (g,h) from sporadic patient 1 (SP1). (a) Low magnification of a lymph node showing an expansion of the paracortical area [haematoxylin and eosin (H&E)]. Congested high endothelial venules and pale-stained dendritic cells are prominent, while lymph follicles with germinal centres are absent. (b) Hyperplasia of the red pulp in the removed enlarged spleen. The white pulp comprises periarteriolar lymphoid sheets (PALS) but is depleted of follicles (H&E). (c–f) Immunohistochemistry of splenic tissue sections. (c) CD3-positive T cells of PALS but (d) no CD20-positive B cells are detected in the splenic white pulp. (e) Disproportional increase in IgA- versus (f) IgG-positive plasma cells in the red pulp. (g,h) Wedge biopsy of the liver showing a nodular pattern resulting from an expansion of hyperplastic hepatocytes adjacent to atrophic liver cell plates consistent with diffuse nodular regenerative hyperplasia. (g) Discrete condensation of reticulin fibres surround small nodules without formation of true fibrous septa (Gomori's reticulin stain). (h) Focal steatosis of hepatocyte nodules with features of non-alcoholic fatty liver disease (H&E).