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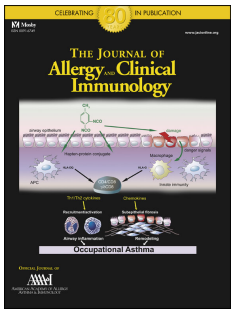

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PII: S0091-6749(16)30097-5

DOI: 10.1016/j.jaci.2016.03.022

Reference: YMAI 12059

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 13 October 2015

Revised Date: 16 February 2016

Accepted Date: 2 March 2016

Clinical and immunological phenotype associated with activated PI3-kinase delta syndrome 2 (APDS2 / PASLI-R1) - A cohort study

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Funding:

A.F., A.D. and S.K. are supported by the European Union's 7th RTD Framework Programme (ERC advanced grant PID-IMMUNE contract 249816).

A.F., A.D., M.C. and S.K. are supported by a government grant managed by the French Agence Nationale de la Recherche as part of the "Investments for the Future" program (ANR-10-IAHU-01).

S.K. is a researcher at the Centre National de la Recherche Scientifique-CNRS (France).

A.F., A.D., M.C. and S.K. are supported by the "Institut National de la Santé et de la Recherche Médicale". S.K. is also supported by the Fondation pour la Recherche Médicale (grant number: ING20130526624), la Ligue Contre le Cancer (Comité de Paris), the Centre de Référence Déficits Immunitaires Héréditaires (CEREDIH) and by the Agence Nationale de la Recherche (ANR-15-CE15-0020) (ANR-PIKimun).

J.R. is supported by the Gebert Rüf Stiftung program "Rare Diseases – New Approaches" (grant no. GRS-046/10), EU-FP7 CELL-PID HEALTH-2010-261387 and EU-FP7

NET4CGD, as well as the Zurich Centre for Integrative Human Physiology (ZIHP), the Gottfried and Julia Bangerter Rhyner Stiftung, and by the Rossi Stiftung.

Se.Ne. is a Wellcome Trust Senior Research Fellow in Basic Biomedical Science (095198/Z/10/Z). S.N. is also supported by the European Research Council Starting grant 260477, the EU FP7 collaborative grant 261441 (PEVNET project), and the National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre.

Sh.No. is partially supported by the grants from MEXT/JSPS, Ministry of Health Labor and Welfare, Ministry of Defense, and the Practical Research for Rare/Intractable Diseases from Japan Agency for Medical Research and Development, AMED.

S.S. is supported by grants form National Institute for Health Research–Leeds Musculoskeletal Biomedical Research Unit (and Leeds Teaching Hospitals Charitable Foundation).

T.C. is supported by National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin, Ireland.

M.L., C.L.L. and H.M. are supported by the Intramural Research Program of NIH, NIAID. C.L.L. was also supported by the Postdoctoral Research Associate (PRAT) Fellowship, NIGMS, NIH.

Abstract:

Background: Activated PI3-kinase delta syndrome 2 (APDS2/PASLI-R1), a recently described primary immunodeficiency, results from autosomal dominant mutations in *PIK3R1*, the gene encoding the regulatory subunit (p85α, p55α and p50α) of class IA PI3-kinases.

Objectives: Review the clinical, immunological and histopathological phenotypes of APDS2 in a genetically defined international patient cohort.

Methods: The medical and biological records of 36 genetically diagnosed APDS2 patients were collected and reviewed.

Results: Mutations within splice acceptor and donor sites of exon 11 of the *PIK3R1* gene lead to APDS2. Recurrent upper respiratory tract infections (100%), pneumonitis (71%) and chronic lymphoproliferation (89%) (including adenopathy (75%), splenomegaly (43%) and upper respiratory tract lymphoid hyperplasia (48%)) were the most common features. Growth retardation was frequently noticed (45%). Other complications were mild neurodevelopmental delay (31%), malignant diseases (28%), most of them being B cell lymphomas, autoimmunity (17%), bronchiectasis (18%) and chronic diarrhea (24%). Decreased serum IgA and IgG (87%), increased IgM levels (58%), B cell lymphopenia (88%) associated with an increased frequency of transitional B cells (93%), decreased number of naive CD4 and naive CD8 but increased number of CD8 effector/memory T cells were predominant immunological features. The majority of patients (89%) received Ig replacement; three patients were treated with rituximab and six patients with rapamycin initiated after diagnosis of APDS2. Five patients died from APDS2-related complications.

Conclusion: APDS2 is a combined immunodeficiency with variable clinical phenotype. Complications are frequent, such as severe bacterial and viral infections, lymphoproliferation and lymphoma similar to APDS1/PASLI-CD. Ig replacement therapy, rapamycin and likely in the near future selective PI3Kδ- inhibitors are possible treatment options.

Clinical Implications: APDS2/PASLI-R1 should be screened in sporadic or autosomal-dominant primary immunodeficiencies associated with lymphadenopathies, growth retardation, high IgM (HIGM-like syndrome), and B cell lymphopenia with an increased percentage of transitional B cells and decreased naïve T cells.

Capsule summary: Comparison of clinical and biological records of 36 genetically diagnosed APDS2/PASLI-R1 patients highlights the severity of the disease and its variable phenotype spectrum.

Key words:

PID

PI3K

p85 alpha

p110 delta

APDS

PASLI

HIGM

adenopathy

immunodeficiency

antibody deficiency

Abbreviations:

APDS: Activated PI3-kinase δ syndrome

PASLI: p110δ-activating mutations causing Senescent T cells, Lymphadenopathy, and Immunodeficiency

PID: primary immunodeficiency

DLBCL: diffuse large B cell lymphoma

HL: Hodgkin lymphoma

CLL: Chronic lymphocytic leukaemia

HLA: Human Leukocyte Antigen

Introduction:

Activated PI3-kinase δ syndrome 2 (APDS2) also named p110δ-activating mutations causing Senescent T cells, Lymphadenopathy, and Immunodeficiency (PASLI-R1) [MIM# 616005] is a primary immunodeficiency (PID) resulting from autosomal dominant mutations in *PIK3R1*, the gene encoding the regulatory subunit (p85α, p55α and p50α) of class IA PI3-kinases (1, 2). Class IA PI3K molecules are composed of a p110 catalytic subunit (p110α, p110β, or p110δ) and a regulatory subunit (p85α, p55α, p50α, p85β, or p55γ) that regulates the stability, cellular localization, and function of p110. The function of class IA PI3Ks is to convert phosphatidylinositol 4,5-bisphosphate into phosphatidylinositol 3,4,5-trisphosphate (PIP3), an important phospholipid secondary messenger. Each of the catalytic subunits can bind to any of the regulatory subunits (3). Expression of the p110δ catalytic subunit is restricted mainly to leukocytes, whereas p110α and p110β are ubiquitously expressed. The widely expressed p85α regulatory subunit is the predominant regulatory subunit in lymphocytes. Mutations in a splice donor site of *PIK3R1* have been shown to cause APDS2 as a result of skipping of exon 11 (coding exon 10), encoding the amino acids 434-475 of p85α. The splicing from exon 10 to exon 12 is in-frame and therefore results in a shortened p85α protein; the p55α and p50α isoforms are similarly affected. The shortened p85α protein is dominantly responsible for hyper-activated PI3Kδ -signaling in T and B lymphocytes (1, 2).

The main clinical and biological findings in the 13 published APDS2 patients reported so far were recurrent respiratory tract infections, lymphoproliferation and antibody deficiency (1, 2, 4, 5). APDS2 resembles APDS1 also named PASLI-CD [MIM# 615513], a PID caused by autosomal-dominant, gain-of-function mutations in *PIK3CD*, the gene encoding the catalytic subunit p110δ, leading to hyper-activated PI3Kδ signaling in lymphocytes (6-8).

In this study we reviewed the clinical, immunological and histopathological features of APDS2 in a genetically defined international cohort of 36 patients.

Patients and Methods:

Genomic DNA from patients presenting with genetically undefined primary antibody deficiency were screened for mutations at the splice sites of exon 11 (coding exon 10) of the *PIK3R1* gene by whole exome sequencing or targeted Sanger sequencing. Medical and biological records of 36 genetically diagnosed APDS2 patients were retrospectively collected and compared using a questionnaire. Patients treated for lymphoma and/or rituximab were excluded from the immunological analysis. The study was performed in accordance with the precepts of the Declaration of Helsinki and local ethical requirements.

Results:

Patient characteristics

In this retrospective analysis, 36 APDS2 patients (15 males) from 31 unrelated families were included, of whom 8 patients were reported previously (1, 2). Five patients died at the age of 12 (P10), 27 (P5 and P28), 30 (P11) and 36 (P20a) years, respectively (Figure 1). Alive patients had a median age of 18 years, (range from 3 to 56 years) at the time of the medical report.

Genetics of heterozygous splice site mutations in *PIK3R1*

The previously described G to A, G to C and G to T nucleotide substitutions at the +1 position of the donor splice site of *PIK3R1* were identified in 42%, 29% and 13% of the patients, respectively (Figure 2). In four patients (13%) novel mutations affecting the +2 position of this donor splice site were identified, a T to A mutation; a T to G substitution, and a TG deletion. In addition, a novel mutation, a G to C nucleotide substitution, at the -1 position of the splice acceptor site of exon 11 of the *PIK3R1* gene was identified (Figure 2). Exon

skipping of the exon 11 encoding the amino acids 434-475 of p85α was demonstrated by mRNA analysis for all novel mutations (Supplemental Figure 1).

Ten patients were familial cases (5 families), but the large majority of patients were sporadic cases. Analysis of DNA from parents was only available for 8 patients from sporadic cases and revealed *de novo* mutations.

Clinical presentation:

Infectious complications

Clinical manifestations of the 36 patients are shown in Figure 3. All presented with early onset recurrent ear, nose and throat (ENT) or broncho-pulmonary infections (median onset 1.7 years of age, range from the first month of life to 10 years of age). Upper respiratory tract infections (otitis media, sinusitis) as well as lower respiratory tract infections (bronchitis and pneumonitis) were present in 100% and 77% of patients, respectively. Mild bronchial wall thickening on chest CT scan and bronchiectasis were noticed in 2 (6%) and 6 patients (18%), respectively, and bronchiectasis was diagnosed with a median age of 13 years (range 4 to 33 years). The most common bacterial respiratory organisms identified were *Haemophilus influenzae* and *Streptococcus pneumoniae*. Chronic conjunctivitis reported in 7 patients progressed in 1 patient to *Staphylococcus aureus*-related periorbital cellulitis (P5b), and in 2 patients to chronic blepharitis (P16 and P27a). Invasive bacterial infections were rare, being only reported in two cases, one patient who presented with *Pseudomonas aeruginosa* septicemia (P20a), and a 12-year-old boy (P10) who developed peritonitis related to infectious perforation of the small intestine leading to septic shock and death. This boy had chronic gastroenteritis associated with *Campylobacter jejuni*, *Salmonella typhimurium* and *Clostridium difficile* infections. Chronic cutaneo-mucosal candidiasis was observed in 3 patients (P5, P25 and P28). Out of 17 patients who received Bacillus Calmette-Guerin (BCG)

vaccination, two (P21 and P26) presented with persistent local skin lesions at the vaccination site. Persistent detection of virus was reported in 36% patients with Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) as the most common. Disseminated lymphadenitis associated with CMV infection was reported in 2 patients, and asymptomatic chronic CMV viremia was detected in 6 patients (17%). Chronic EBV viremia was detected in 8 patients (22%); reported in 4 patients in combination with EBV-associated lymphoproliferative disease and in 4 patients as asymptomatic chronic EBV viremia. Severe varicella zoster virus (VZV) infections requiring hospitalization occurred in 2 patients (P21 and P26). One patient developed hydrocephalus following measles meningitis (P22). Two patients presented with localized molluscum contagiosum (P17 and P27a) and 1 patient with warts (P22) indicating pox virus and papilloma virus infections, respectively. Chronic viral hepatitis was reported in three patients, related to either hepatitis B (P11, P20a) or C (P5) virus infection. Except chronic *Giardia intestinalis* in one patient (P5), and ocular toxoplasmosis in another (P20a) no other parasitic infections were reported in our patient cohort.

Lymphoproliferation

Thirty-two of 36 patients (89%) showed persistent (>6 months) benign lymphoproliferation either as chronic lymphadenopathy, splenomegaly, ENT or gut infiltration (Figure 3). Lymphadenopathy and splenomegaly typically began in childhood. Lymphadenopathies mentioned in 75% of the patients were variable in size, from mild (1-3cm) in 18 of 36 of patients (50%) to large in 9 patients (25%) (3-5cm, n=7; >5 cm, n=2). 15 patients (43%) developed splenomegaly of variable size (Figure 3). Hepatomegaly developed in 8 patients (22%). Nodular lymphoid infiltration of the gut was reported in 8 patients (24%) and was associated with chronic diarrhea and/or malabsorption. Severity of ENT infiltration was variable, ranging from ENT chronic lymphoid hyperplasia without the need for surgical

interventions in 3 patients (11%), to adenoidectomies and/or tonsillectomy in 7 patients (26%) and to multiple surgical resections in 3 patients. One 6-year-old patient required multiple surgical interventions, including maxillary antrostomies, multiple adenoidectomies, tonsillectomies and reductions of basilingual tonsils. The patient subsequently developed postoperative pharyngeal stenosis, requiring 3 endoscopic dilations, which were inefficient leading to tracheotomy.

Tonsil biopsies from P1 and P2 were available. As shown in Figure 4 and Supplemental Figure 2 (both presented identical abnormalities as compared to age-matched controls): prominent T cell hyperplasia and small B cell follicles were noticed. Germinal centers were small and ill-defined, with very few IgD-positive mantle cells. Large B cells in the interfollicular area were numerous and IgM-positive cells that are usually localized in germinal centres were scattered within the T cell zone. In addition, an important hyperplasia of PD1-positive T cells was present both in germinal centre and in extrafollicular areas. The frequency of scattered EBV- and/or CMV-positive cells present in the patients' biopsies were comparable to those observed in control biopsies and not consistent with EBV- and/or CMV-driven pathologies.

Lymphoma

Ten patients (28%) developed malignant diseases (Table 1 and Figure 1B) at a median age of onset of 23 years (6 to 40 years). The cumulative risk of developing lymphoid malignancy at the age of 40 years was calculated to be 78% (Figure 1B). Classical Hodgkin Lymphoma (CHL) was diagnosed in 5 patients (14%). Diffuse large B cell lymphoma (DLBCL) was diagnosed in 4 patients (11%) and marginal zone B cell lymphoma in 2 patients (6%). Three patients developed multiple lymphomas. One patient (P12) firstly developed at the age of 14 years a nodular sclerosis CHL, treated by chemotherapy and at the age of 27 years a DLBCL,

treated by intensive chemotherapy and autologous hematopoietic stem cell transplantation (HSCT). Another patient (P20a) had 2 EBV-positive nodular sclerosis CHL at 14 and 35 years of age and a marginal zone B cell lymphoma at 19 years of age. Her brother (P20b) presented also with CHL when he was 8 years old. Overall, 4 patients died of lymphoma at the ages of 27 (P5 and P28; DLBCL), 30 (P11; CHL) and 36 (P20a; CHL) years, respectively. Chronic lymphocytic leukaemia (CLL) developed in one patient (P27a) at 40 years of age. No other malignancy has been reported, except a papillary neoplasm in both breasts in a female patient.

Autoimmunity and immune dysregulation

Six patients (17%) developed autoimmune complications. Two patients had thrombocytopenic purpura during childhood. One patient developed autoimmune hemolytic anemia post chemotherapy for lymphoma and one patient developed Evans syndrome associated with CLL. An insulin-dependent diabetes was diagnosed in one patient. Two suffered from chronic arthritis, and one patient developed autoimmune hepatitis. In addition, three patients presented with chronic eczema.

Immunological features:

The patients' main immunological characteristics are summarized in Figure 5A-H (Immunological data for individual patients are provided in Online Repository tables E1-4). The majority of patients presented with decreased serum IgG and IgA levels before onset of Ig replacement therapy (87%). Increased IgM levels were observed in most patients (58%) but not all since 26% presented with a normal level and 16% with a decreased level, before any treatment. IgM levels decreased in 5 patients and increased in 2 patients over 2-12 years after onset of Ig replacement therapy. One patient (P3a) had increased IgG and IgM but decreased

IgA levels; one (P28) had low IgA but normal IgG and IgM levels and one (P4a) had increased IgA, decreased IgG and normal IgM levels.

The majority of patients (88%) presented with B cell lymphopenia worsening within 1 to 19 years (Figure 5D and Supplemental Figure 3). Transitional B cells were increased in frequency in 14 out of 15 patients (93%) who had a suitable number of CD19-positive cells for analysis. Total CD3 T cell counts were normal in 74% of patients (Supplemental Figure 4), CD4 T cell counts were normal in 67% of patients while CD8 T cell counts were increased in 52% of patients and remained stable over time. An inverted CD4/CD8 ratio (<1.0) was found in 82% of patients. When extended naive/memory T cell phenotype analysis was performed, the increased CD8 T cell number appeared as resulting from expanded CD8 T cells population with an effector/memory phenotype. Nearly all patients analyzed presented with a low number of naive CD4 T cells (CD31+CD45RA+/CD4+; 71% of patients) and naive CD8 T cells (CCR7+CD45RA+/CD8+; 100% of patients) worsening over time (Figure 5F, G and Supplemental Figure 5A, B).

Non-immunological features

Growth impairment (-2SD of height) was found in 14 out of 31 patients (45%), a feature not always related to chronic diarrhea since it was absent in 9 of them. Height and weight were similarly affected since body mass index was within the normal range in all but 2 patients (min = -2.8 SD; max = +3.3 SD; median = -0.7 SD). Microcephaly was reported in 2 patients. Neurodevelopmental delay presented as mild cognitive impairment or learning disabilities was reported in 9 patients (31%). For one patient, extensibility of joints and increased glucose levels in the blood were also reported. Liver cysts and polycystic kidneys were reported in one patient each.

346

347 **Treatment**

348 Twenty-two patients received various antibiotic prophylaxis (trimethoprim/sulfamethoxazole
349 or azithromycin). The majority of patients (89%) received Ig replacement therapy (median
350 age at onset of treatment 5 years; range 1 to 35 years). Five patients were treated with steroids
351 because of autoimmune cytopenia (n= 2) or lymphoproliferation (n= 3). Three patients were
352 treated with rituximab to treat lymphoproliferation (n= 2) or autoimmune hemolytic anemia
353 (n= 1). Three patients were splenectomized, two for autoimmune cytopenia and one as a
354 diagnostic procedure of massive splenomegaly. Immunosuppressive drugs for digestive tract
355 disease were given in 3 patients in different combination (azathioprine, mycophenolate
356 mofetil, methotrexate and infliximab). Episodes of lymphomas were treated conventionally
357 with chemotherapy associated in some cases with radiotherapy and in three patients with
358 autologous HSCT. Allogeneic HSCT from a HLA matched (10/10) unrelated donor was
359 performed in one patient (P27b) because of molecular diagnosis, recurrent infections and the
360 family history. The conditioning regimen consisted in treosulfan 42 g/m^2, fludarabine 150
361 mg/m^2 and alamtuzumab. Five months post HSCT he was alive and well with 100% donor
362 chimerism and no sign of GVHD. Since the diagnosis of APDS2, 6 patients were started on
363 rapamycin treatment. The time of follow up after onset of rapamycin treatment was too short
364 to evaluate treatment efficacy for 4 patients. Two APDS2 patients were doing well on
365 rapamycin treatment. For both patients significant reduction of lymphoproliferation was
366 reported.

367

368 ## Discussion

369 Our retrospective analysis comparing clinical features of APDS2/PASLI-R1 patients
370 indicated a highly heterogeneous clinical phenotype with recurrent ENT and broncho-

pulmonary infections during early childhood as the most common clinical manifestation. Chronic benign lymphoproliferative complications with various degrees of severity manifesting as adenopathies, spleno- or hepatomegaly were observed. Persistent EBV and/or CMV viremia were detected in several patients, indicating impaired control of viral infections. Predominant biological parameters included HIGM features, B cell lymphopenia associated with increased frequency of transitional B cells, decreased naive CD4 and CD8 T cell numbers and increased cell number and frequency of CD8 effector/memory T cells. B cell lymphoma, especially CHL, DLBCL and marginal zone B cell lymphoma were frequently reported in our cohort, indicating the oncogenic character of these *PIK3R1* splice-site mutations. Non-infectious and immunological manifestations noted in our APDS2 cohort of patients were growth retardation and mild neurodevelopmental delay.

Overall our work underscores the conclusion that APDS2 shares similarities with APDS1. Both syndromes present a predominant antibody deficiency frequently presented as an hyper-IgM like syndrome associated to a progressive B and naive T cell lymphopenia and massive lymphoproliferation. The phenotypic heterogeneity of APDS2 patients, similar to that observed in APDS1, may be related to the patient's history of infections, environmental factors, and/or the presence of modifier genes.

However, in contrast to APDS1 (Coulter et al., submitted), histological analysis revealed in the two available tonsil biopsies a reduced size of germinal centers. Although we cannot exclude that this observation could be due to the heterogeneous spectrum of the disease since only a limited number of biopsies were available, it might suggest that deletion of exon 11 of the *PIK3R1* gene affects not only p110δ but other catalytic subunits of class IA PI3Ks.

Increased or normal IgM together with decreased IgG and IgA serum levels and B cell lymphopenia associated with increased frequency of transitional B cells were frequently

observed in APDS2 patients and can be explained by an intrinsic B cell defect leading to enhanced differentiation of APDS2 B lymphocytes into short-lived IgM producing plasmablasts as reported for PTEN deficient murine B cells (17). Our histological analysis identifying numerous large IgM positive B cells located in the interfollicular area further supports this hypothesis. Moreover, our histological analysis indicated that hyperactive PI3K signalling interferes with the germinal centre structure likely inhibiting Ig class switch recombination. Impaired Ig class switch recombination as a cause of disturbed germinal centre architecture was indeed recently described in a murine model analysing hyperactive PI3K signalling in germinal centre B lymphocytes (18). The B cell lymphopenia in the blood of APDS2 patients could be explained by disturbed migration since B cells are proliferating in the lymphnodes as indicated by our histological analysis.

The major complication of APDS2 patients (as well as of APDS1 patients) is the development of B cell lymphoma (9/36; 25%) (8, 9). Predisposition of APDS2 (as for APDS1) to B-cell lymphomagenesis could be due to several immunological abnormalities, such as a defective T-cell–mediated immune surveillance or uncontrolled B cell activation and proliferation or both. As the histopathological analysis indicated an important hyperplasia of PD1-positive T cells, aberrant T_{FH} cell function could be considered as an additional factor for promoting survival of neoplastic B cells, as previously suggested for T_{FH} cells present within the microenvironment of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and follicular lymphoma (FL)(10). Oncogenic potential of *PIK3R1* mutations have been previously suggested by the presence of somatic mutations in *PIK3R1* in Burkitt Lymphomas (11) and in endometrioid and colon cancers (12) affecting the amino acid residues 437-475, encoded by exon 11 (coding exon 10) of the *PIK3R1* gene. Moreover, in the somatic mutations in cancer (COSMIC) project, mutations affecting the + 1 position (G to A and G to T) and +2 position (T to C and T to G) at the same splice-acceptor site of the *PIK3R1* gene

found mutated in APDS2 have been recently annotated, a strong argument in favor of the oncogenic character of these APDS2 splice-site mutations. These somatic mutations were found in carcinoma located in ovary, large intestine, stomach and malignant melanoma underlining the possible oncogenic potential of those mutations not only for B cell lymphoma but for other cell types, suggesting an impairment of the *PIK3R1* gene encoded regulatory subunits not only on p110δ (PI3Kδ) activity. Growth impairment, extensibility of joints and increased glucose levels in the blood reported for one patient might reflect deregulated p110α (and/or p110β) activity. More research will be needed to characterize possible effects of the mutant $p85\alpha^{\Delta 434\text{-}475}$ protein on the different catalytic p110 subunits in other cells (non-lymphoid lineage cells) which could be hidden by the predominant immunological phenotype. Of note, heterozygous non-synonymous germline mutations located especially within the C-terminal part of p85α (downstream of amino acid 475) result in a rare autosomal dominant multisystem disease called SHORT syndrome described to be due to loss of PI3K-activity (13-15). SHORT syndrome patients present with short stature (S), hyperextensibility of joints or hernia (inguinal) or both (H), ocular depression (O), Rieger anomaly (R), and teething delay (T).

Allogeneic HSCT for APDS2 was recently reported for one case (5). Herein we describe a second successful case similarly to the 8 of 11 successful cases of allogeneic HSCT for APDS1 (Coulter et al., submitted). Thus allogeneic HSCT appears to be a treatment option for severely affected APDS2, especially in the light of the increased risk of lymphoma development (Table 1 and Figure 1B), although no prognostic marker for the development of lymphoma has been identified so far.

Most patients have received Ig replacement therapy since infancy to reduce the infection incidence. Since the diagnosis of APDS2, six patients were started on long-term rapamycin treatment based on the knowledge that the serine/threonine kinase mammalian target of

rapamycin (mTOR) is activated by PI3K signaling, and rapamycin treatment was reported to be beneficial in APDS1 patients ((7) and personal observation). For two APDS2 patients in our cohort rapamycin treatment was beneficial. For one patient treatment led to disappearance of chronic conjunctivitis and normalization of tonsil size and for the other patient to reduced lymph node, liver and spleen sizes; however, the impact of this treatment on lymphocyte cell numbers and antibody titers and over a longer time period has to be further investigated. Evaluation of the efficacy of rapamycin treatment on the other APDS2 patients in our cohort was not possible due to the short treatment period. Although continuous rapamycin treatment might turn out to be very beneficial for APDS2 patients, it bears the risk of unwanted side-effects outside the immune system (16). Since the hyper-activated PI3K signaling in APDS2 lymphocytes is mediated by the catalytic p110δ-subunit (1, 2), treatment with p110δ-specific inhibitor could offer a new treatment prospective with possibly higher efficiency and less unwanted side-effects.

Overall our study indicates that the splice-donor and splice-acceptor sites of the exon 11 (coding exon 10) of the *PIK3R1* gene should be sequenced in sporadic or autosomal-dominant primary immunodeficiencies associated with lymphadenopathies, growth retardation, antibody deficiency, especially HIGM, B cell lymphopenia with an increased percentage of transitional B cells as well as naïve CD4 and naïve CD8 T cell lymphopenia.

Finally, our study also indicates the need for further prospective, large-cohort studies of APDS2 in order to identify clinical or laboratory biomarkers that predict disease severity and to document the impact of different treatment options.

Acknowledgments:

We thank Manon Fasquel, Monique Forveille, Stéphanie Ndaga, Nathalie Lambert, Virginie Grandin, Ioana Morel, and Aminata Diabate for technical and Dr. Masao Kobayashi, Dr.

Satoshi Okada, Dr. Yujin Sekinaka, Dr. Yuki Tsujita, Dr. Alessandro Plebani, Dr. Akira Shimada, Dr. Kunio Hashimoto and Dr. Junnichi Ueyama for medical support. We also thank Ms. Susan Price and Drs. Ying Wang, Koneti Rao, Elif Karakoc, and Tamara Pozos.

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529

Figure Legends

Figure 1. Overall survival and lymphoma-free living of APDS2 patients.

A) Overall survival of APDS2 patients from the cohort. (B) Cumulative risk of developing lymphoma according to age: the time of lymphoma-free life in APDS2 patients is herein depicted.

Figure 2. Confirmed heterozygous mutations in the *PIK3R1* gene of APDS2 patients.

Frequency and number of patients carrying indicated mutations are presented. Mutations present in several patients from one family were counted as one mutation.

Figure 3. Main clinical characteristics of APDS2 cohort.

Shown is the percentage of patients, which presented with indicated clinical features. ENT: ear, nose throat; neurodev. delay: neurodevelopmental delay.

Figure 4. Main clinical complications and biological features of APDS2 patients.

A) Clinical features of APDS2 patients. B) Biological features. * median age at medical report of alive patients, red: affected; light yellow: unaffected and boxes with a diagonal: unknown. C) Variability of lymphoproliferation; light yellow: unaffected and boxes with a diagonal: unknown; dark yellow: ENT chronic lymphoid hyperplasia without the need of surgical interventions, lymphadenopathies with lymph node sizes from 1-3cm; orange: adenoidectomies and/or tonsillectomy, lymph node sizes from 3-5cm; red: multiple surgical resections, lymph node sizes larger than 5 cm. Splenomegaly was graded by its size: light yellow if it was not present, dark yellow up to half distance between coastal margin and ombilicus, dark orange up to ombilicus, or red above ombilicus.

Figure 5. Histological features of APDS2 patient's tonsil biopsies.

A to E: APDS2 patient. A' to E': Control. All pictures are at the same magnification: x10; GC: germinal centre, MZ: mantle zone. B-cell Follicles are small (A: follicles defined by circles, B, C, D) with a few CD20+ B-cells (inset CD20 staining A) compared to control (Inset CD20 staining A') and associated with prominent CD3+ T-cell hyperplasia (B and B', anti-CD3 staining) compared to control. PD1 staining underlines the important hyperplasia of germinal centre- and extrafollicular-PD1+ T-cell (C) compared to control (C'). Germinal centres are ill-defined (A, B, C) and IgM+ cells usually localized in the germinal centre (D', control) are scattered in the T-cell zone (D). Only few residual IgD+ mantle cell zone cells are present (E) compared to control (E').

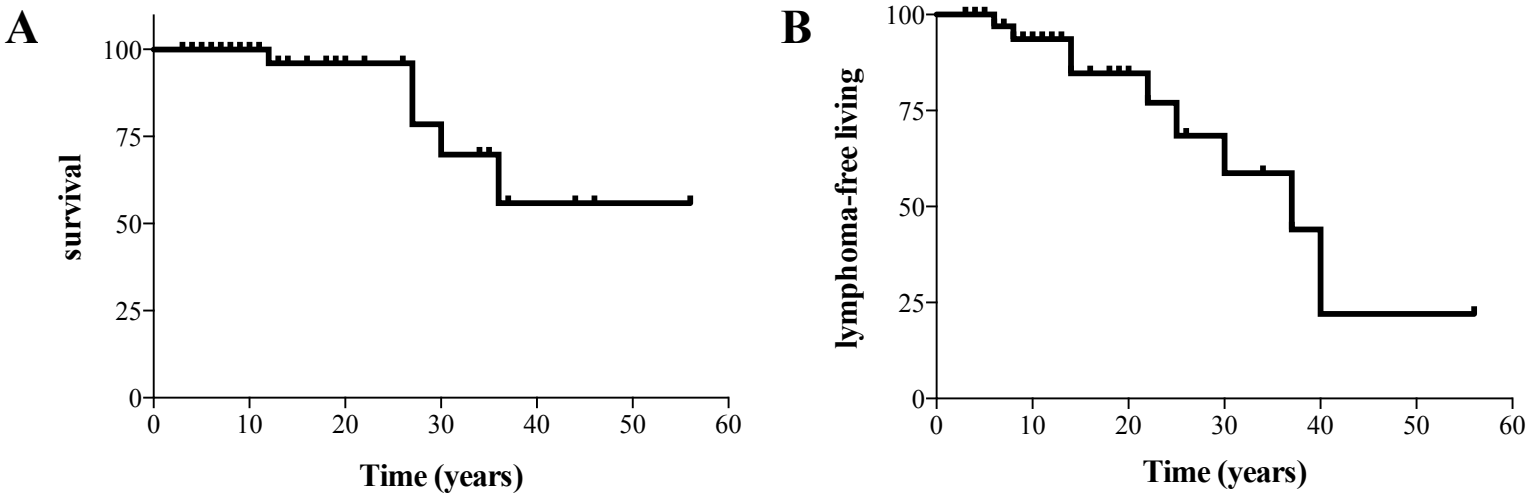
Figure 6. Immunological features of APDS2 patients.

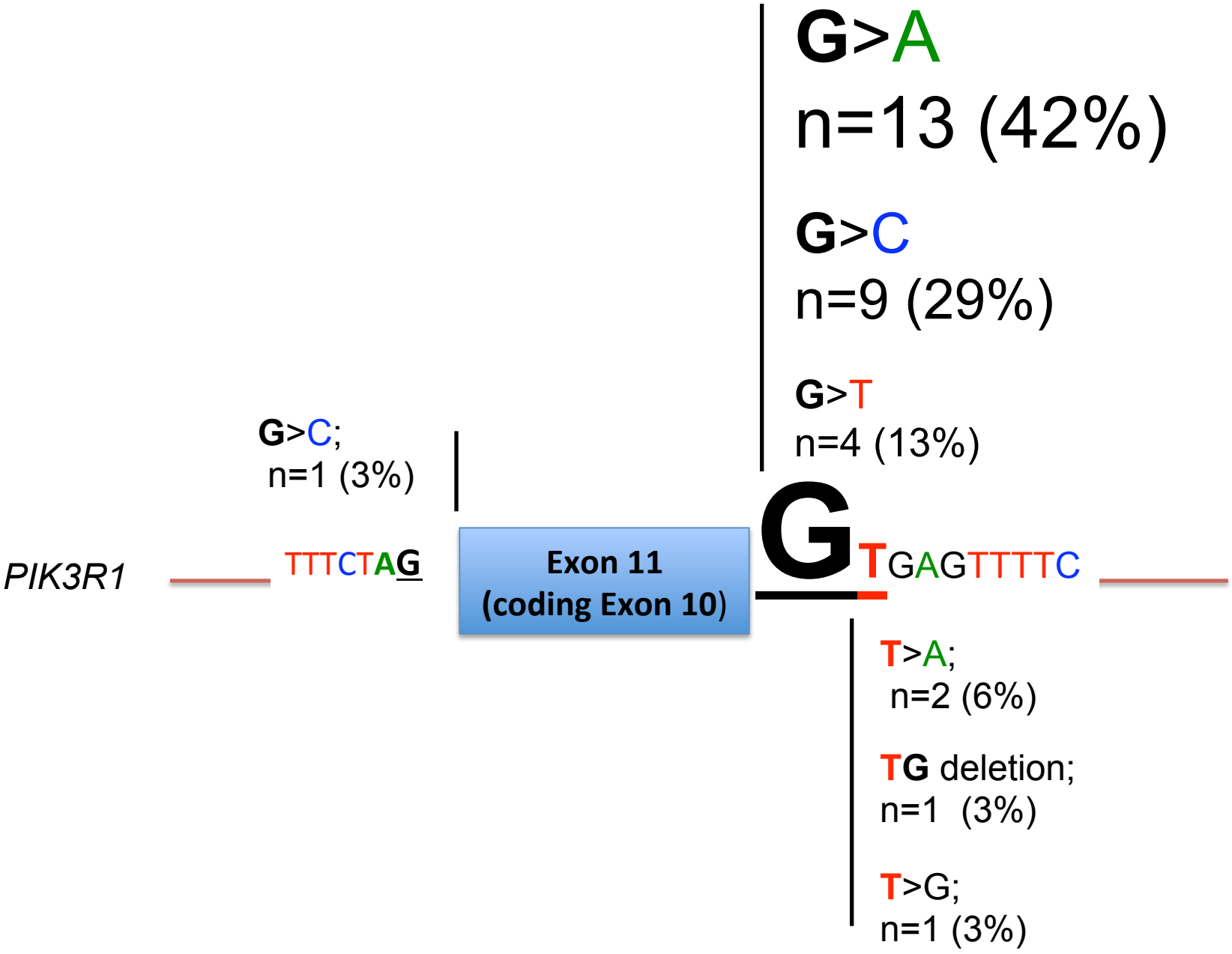
A) IgG, B) IgA and C) IgM level and D) B cell number of APDS2 patients before onset of Ig replacement or other therapies. E) CD4 F) CD4 naive, G) CD8, H) CD8 naive T cell subsets of APDS2 patients. E) and G) before onset of Ig replacement or other therapies and F) and H) at last evaluation. Solid line: lower reference value; dashed line: upper reference value.

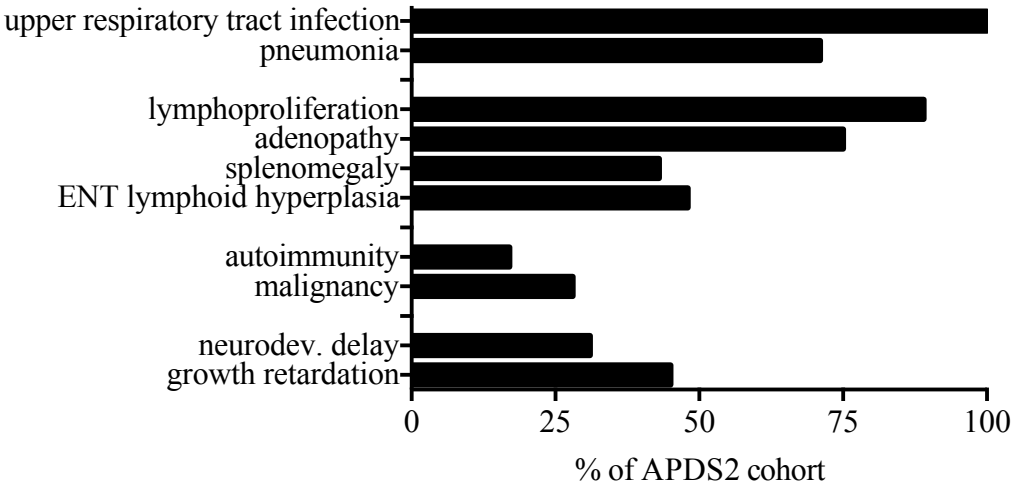
Table 1 : Malignant diseases

Patients	Age at PID diagnostic (yrs)	Age at onset of cancer (yrs)	Type of cancer	Dead/ Alive
P5	4	25	DLBCL	Dead
P11	22	30	CHL	Dead
P12	Infancy	14/27	CHL/DLBCL	Alive
P19	9	6/11	DLBCL/MALT	Alive
P20a	36	14/19/35	CHL/MZL/CHL	Dead
P20b	8	8	CHL	Alive
P22	6	30	Breast papillary neoplasm	Alive
P23b	Infancy	37	CHL	Alive
P27a	31	40	CLL	Alive
P28	5	22	DLBCL	Dead

DLBCL : Diffuse large B cell Lymphoma; CHL: Classical Hodgkin Lymphoma; MALT: Mucosa-associated lymphoid tissue Lymphoma; CLL: Chronic Lymphocytic Leukaemia; MZL: Marginal zone B cell Lymphoma


A
survival
100
75
50
25
0
0
10
20
30
40
50
60
Time (years)
B
lymphoma-free living
100
75
50
25
0
0
10
20
30
40
50
60
Time (years)


G>A
n=13 (42%)
G>C
n=9 (29%)
G>T
n=4 (13%)
G>C;
n=1 (3%)
PIK3R1
TTTCTAG
Exon 11
(coding Exon 10)
GTGAGTTTTC
T>A;
n=2 (6%)
TG deletion;
n=1 (3%)
T>G;
n=1 (3%)


upper respiratory tract infection
pneumonia
lymphoproliferation
adenopathy
splenomegaly
ENT lymphoid hyperplasia
autoimmunity
malignancy
neurodev. delay
growth retardation
0
25
50
75
100
% of APDS2 cohort

A) Clinical features of APDS2 patients

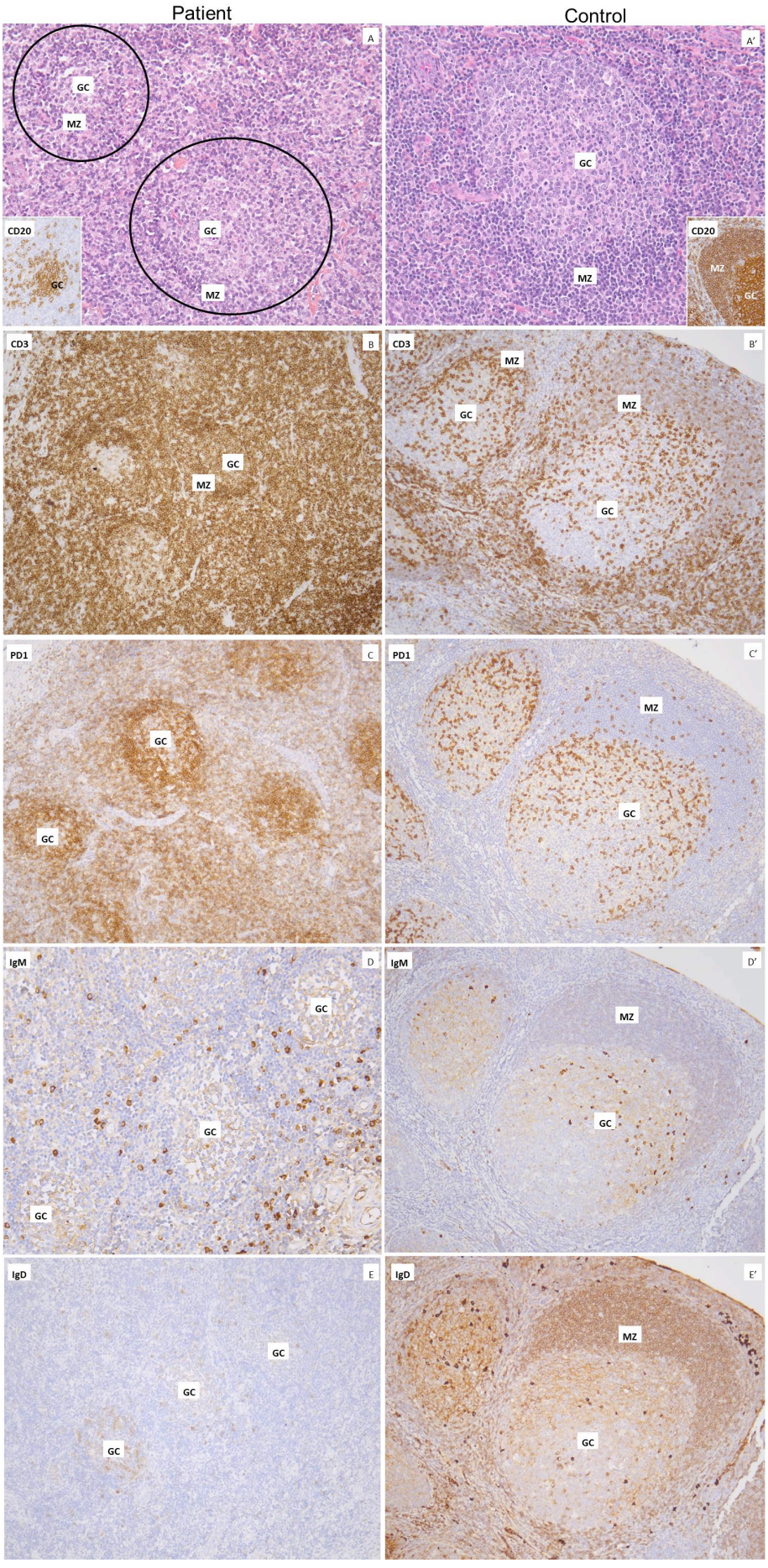
Patient	%	1	2	3 a	3b	4 a	4b	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20a	20b	21	22	23a	23b	24	25	26	27a	27b	28	29	30	31
Age at medical report (yrs)	18*	13	7	37	5	56	19	27	26	20	6	11	12	30	35	19	16	34	9	11	14	18	36	26	7	34	18	44	18	8	4	46	16	27	10	3	22
Upper respiratory infections	100																																				
Pneumonia	71																																				
Bronchiectasis	18																																				
Autoimmunity	17																																				
Chronic diarrhea	24																																				
Adenopathy	75																																				
Splenomegaly	43																																				
Malignant disease	28																																				
Neurodevelopmental delay	31																																				
Growth retardation	45																																				
Dead	14																																				

B) Biological features

Patient	%	1	2	3 a	3b	4 a	4b	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20a	20b	21	22	23a	23b	24	25	26	27a	27b	28	29	30	31
Increased IgM	58																																				
Decreased IgA/IgG	87																																				
EBV chronic replication	22																																				
CMV chronic replication	17																																				
Inverted ratio CD4/CD8	82																																				

C) variability of lymphoproliferation

Patient	1	2	3 a	3b	4 a	4b	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20a	20b	21	22	23a	23b	24	25	26	27a	27b	28	29	30	31
ENT lymphoid hyperplasia																																				
Splenomegaly																																				
Adenopathy																																				


Patient
Control
A
GC
MZ
CD20
GC
GC
MZ
A′
GC
MZ
CD20
MZ
GC
CD3
B
GC
MZ
CD3
B′
MZ
GC
MZ
GC
PD1
C
GC
GC
PD1
C′
MZ
GC
IgM
D
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IgD
E
GC
GC
GC
IgD
E′
MZ
GC

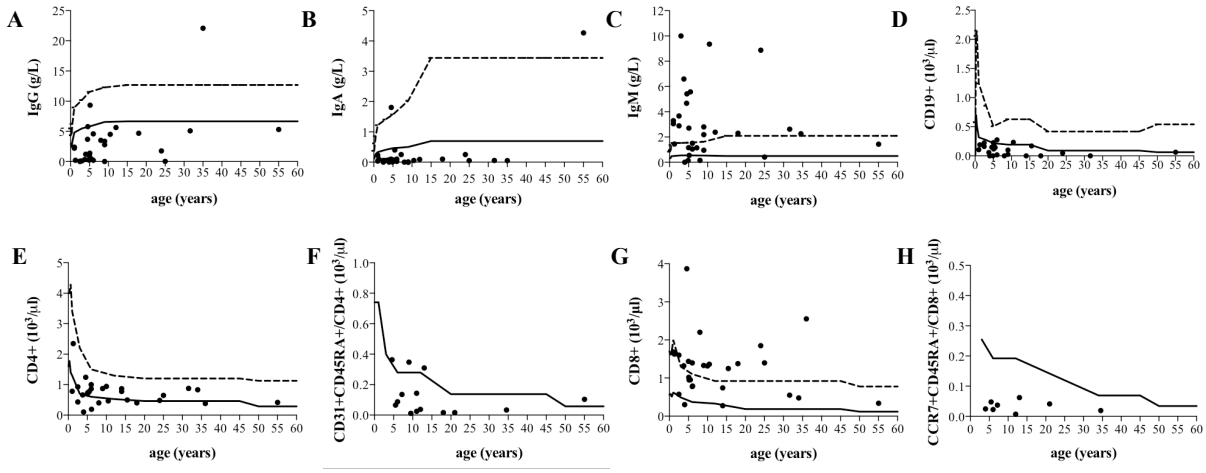

A
IgG (g/L)
age (years)
B
IgA (g/L)
age (years)
C
IgM (g/L)
age (years)
D
CD19+ (10³/µl)
age (years)
E
CD4+ (10³/µl)
age (years)
F
CD31+CD45RA+/CD4+ (10³/µl)
age (years)
G
CD8+ (10³/µl)
age (years)
H
CCR7+CD45RA+/CD8+ (10³/µl)
age (years)

Table E1. B lymphocyte subsets and Ig serum levels at initial assessment

Patient	Age (yrs)	IgG (g/L)	IgA (g/L)	IgM (g/L)	CD19+ (/µL)	CD19+/ CD21+ CD24++ (%)	CD19+/ CD38+ IgM+ (%)	Memory B cells (%)	Switched memory B cells (%)	MZB cells (%)
P1	**1**	2.2	0.23	3.3					11	
P2	**2.5**	0.07	0.06	3.67	162	26		8	25	45
P3a	**34**	22	0.05	2.26						
P3b	**2.5**	0	0	2.89	224			8	41	
P4a	**NA**									
P4b	**5.5**	3.64	<0.07	1.66	144					
P5	**4**	1.2	0	0	0					
P6	**6**	0.2	0.03	1.5	20					
P7	**NA**									
P8	**5.1**	1.4	0.07	2.7	120			11	5	6
P9	**3.8**	0.33	0.07	6.6	60			37	26	11
P10	**1.3**	0.2	0.1	1.44	192					
P11	**NA**									
P12	**NA**									
P13	**12**	5.64	0.09	2.38						
P14	**6**	4.6	0.1	1.05	277					
P15	**24**	1.75	0.254	8.87	48					
P16	**9**	2.8	0	> 2.8	100					
P17	**10.5**	4.5	0.06	9.35	233					
P18	**4.5**	3.68	0.1	4.68						
P19	**17**	3.37	0	2.19				2.5	5.1	
P20a	**NA**									
P20b	**8**	3.5	0	0.15						
P21	**7**	0.16	0.25	1.15						
P22	**NA**									
P23a	**NA**									
P23b	**NA**									
P24	**NA**									
P25	**4.9**	0.24	0.01	0.14	0.52					
P26	**4.6**	5.79	1.81	5.42	149		15.24	2.3	1.3	0.2
P27a	**31.6**	5.09	0.06	2.62	250					
P27b	**3**	0.1	0.06	10						
P28	**5.2**	9.34	0.06	0.55	180					
P29	**5**	0.9	0.07	1.16	228		65.8	0.8	0.5	0.3
P30	**1**	2.38	0.04	3.06	107	70.1		9.2		3.9
P31	**18**	4.7	0.1	2.3	55			14	0.0	

Table E2. B lymphocyte subsets and Ig serum levels at later assessment

Patient	Age (yrs)	IgG (g/L)	IgA (g/L)	IgM (g/L)	CD19+ (/µL)	CD19/ CD21+ CD24++ (%)	CD19+ / CD38++ IgM++ (%)	Memory B cells (%)	Switched memory B cells (%)	MZB cells (%)	Treatment with Rituximab
P1	12	16.97 (s)	0.11	1.91	108	11	12	22	13	2	7.5 years post rituximab
P2	5.5	14.6 (s)	0.05	1.73	105	39	39	8	3	2	
P3a	34.6	22 (s)	0.05	2.26	119	11		64.5	42	34	
P3b	4	8.28 (s)	0.05	4.81	120	33	31	9	6	2	
P4a	55	5.3	4.27	1.43	64		2	39	22	17	
P4b	18.5	0.3	0.04	10.63	58		5	50	45	13	
P5	26*	5 (s)	0	0	0						
P6	25	9.27 (s)	0.04	0.04	10						
P7	22				38			14			
P8	6	6.84 (s)			95		23	14	7	4	
P9	11	9.06 (s)	0.07	2.81	30			21	12	9	
P10	NA										
P11	25	0.03	0.05	0.42							
P12	NA										
P13	18	10.08 (s)	0.05	5	81	50.8		25.5	18.6	6.9	
P14	16.5	5.68 (s)	0.1	0.1	74						
P15	NA										
P16	9.5	5.97 (s)	0	0.77	54	9.2	42	27	19	9.1	
P17	11	8.7 (s)	0.06	10.5	160	19.2	45.7	8.5	3.1	4.2	
P18	13	11.26 (s)	0.05	1.85	19	20		18	93		
P19	27						9.23	2.5	50	33.3	
P20a	NA										
P20b	26***	5.8 (s)	0	0.16	0						
P21	7.2	0.16	0.25	1.15	64	40	37	0.6	0.6	3	
P22	33.7$	15.11 (s)	0.05	16.46	122						
P23a	16	9.5 (s)	0	0	0						1 year post rituximab
P23b	NA										
P24	14				13.8						
P25	6.1	4.45 (s)	0.13	0.003							
P26	4.6	5.79	1.81	5.42	149		15.24	2.29	1.3	0.2	
P27a	44.4$	10.3 (s)	0.06	0.19	1						
P27b	15	7 (s)	0.06	0.05	32			15	2	12	
P28	25*	16.3 (s)	0.06	0.42	33	68		28	9.8	13.7	2 years post rituximab
P29	9	13 (s)	0.06	0.63			40.5		1.9	4.3	
P30	2.2						9.23	2.5	50	33.33	
P31	NA										

*<2 years after post chemotherapy; ** > 10 years after chemotherapy and radiotherapy for HL; *** > 10 years after chemotherapy for lymphoma; $ on immunosupressive drugs; (s) on immunoglobulin replacement

Table E3. T lymphocyte subsets at initial assessment

Patients	Age (yrs)	CD3+ (/µL)	CD4+ (/µL)	CD8+ (/µL)	CD16+CD56+ (/µl)
P1	1				
P2	2.5	1512	432	576	126
P3a	34	1394	833	476	
P3b	2.5	2688	928	1600	224
P4a	NA				
P4b	5.5	1878	771	953	285
P5	4	400	100	300	100
P6	6	2500	1000	1400	350
P7	15.5	2035	496	1251	
P8	5.1	2160	730	1020	350
P9	3.8	2210	660	1320	120
P10	1.3	4363	2349	1630	288
P11	NA				
P12	NA				
P13	12				
P14	6	1793	861	780	633
P15	24	2560	482	1850	134
P16	9	2350	870	1330	190
P17	10.5	2100	470	1363	2350
P18	4.5	1900	779	741	
P19	17				
P20a	36				
P20b	NA				
P21	NA				
P22	6				
P23a	16	2661	2233	942	430
P23b	NA				
P24	NA				
P25	4.9	1144	195	789	115
P26	4.6	5357	1243	3868	764
P27a	31.6	1200	880	550	210
P27b	NA				
P28	5.2	1580	730	950	200
P29	5	2187	696	1439	285
P30	1	2769	781	1687	835
P31	18	1800	400	1380	320

Table E4. T lymphocyte subsets at later assessment

Age (yrs)	CD3+ (/µL)	CD4+ (/µL)	Naive CD4 CD31+ CD45RA/ CD4+ (%)	Naive CD4 CD31+ CD45RA/ CD4+(/µL)	CD8+ (/µL)	Naive CD8 CCR7+ CD45RA+ /CD8+(%)	Naive CD8 CCR7+ CD45RA+/ CD8+(/µL)	CD16+ CD56+ (/µL)	Senescent T cell (%)
12	1440	540	7	38	702	1	7		19.5
5.5	957	330	20	66	341	14	48	55	16
34.6	1394	833	4	33	476	4	19	187	16.3
4	3432	780			2457		25	156	8.89
55	764	420	24	103	337			356	
18.5	2107	617	7	42	1330	5	63	138	
26*	280	20	0	0	260			20	
25	1940	770			1140			240	
22	1714	452	5	23	1036	4	41	132	23.4
6	11558	513	17	87	760	2	15	228	21.3
11	1440	530	22	140	750			30	
NA									
25		650			1400				
NA									
18	704	200	8.5	17	415			1031	
16.5	2067	886			1187			14	
NA									
9.5	2155	488	3.2	12	1384			56	
11	2930	530	4.7	25	2390			1650	
13	2040	1032	30	310	888	7	62	312	26.4
NA									
36**	3015	385	4	16	2554	1	23	157	
26***	2600	400	0.5	2	2200	0	0	100	
7.2	1312	432	31	135	512	7	37		
33.7$	4488	1079			3210				
16	2233	942			1318			430	
NA									
14	1242	869			276				
NA									
4.6	5357	1243	29	363	3868			764	
44.4$	455	228			218			6	
15	965	297	35	103	619			283	
25*	3302	404			2898			370	
9	3839	2614	13	347	1225			124	
NA									
NA									

*<2 years after post chemotherapy; ** > 10 years after chemotherapy and radiotherapy for HL; *** > 10 years after chemotherapy for lymphoma; $ on immunosupressive drugs; (s) on immunoglobulin replacement

Clinical and immunological phenotype associated with activated PI3-kinase delta syndrome 2 (APDS2 / PASLI-R1) - A cohort study

Online repository Materials

Supplemental Figure 1. Novel splice acceptor and splice donor mutation at exon 11 (coding exon 10) of the PIK3R1 gene lead to exon skipping.

A) RT-PCRs with primers flanking exon 11 of the *PIK3R1* gene with RNA extracted from patients (P19: PBLs; P8: fibroblasts and P10: T cell blasts) and healthy individuals (control 1,2,3: PBLs; control 4: fibroblasts; control 5: T cell blasts). P19: *de novo* mutation at splice acceptor site (GRCh38; NM181523.2; C.1300 -1 position; G to C); P8: *de novo* mutation at splice donor site (GRCh38; NM181523.2; C.1425 +2 position T to G) and P10: 2 nt deletion at splice donor site (GRCh38; NM181523.2; C.1425 +2,3 position; TG deletion).

B) Sequencing chromatogram showing the skipping of exon 11 (coding exon 10). Sequencing was performed with PCR products amplified from cDNA from P28. P28: *de novo* mutation at splice donor site (GRCh38; NM181523.2; C.1425 +2 position T to A).

Supplemental Figure 2. Large B-cells in the interfollicular area are in cycle.

APDS2 patient's tonsils histology showed numerous Ki67 positive cells outside the positive germinal centre (A x10) in comparison to a control (A' x10). Double staining (B, CD20: brown, Ki67: red; x40) showed that large B-cells in the interfollicular area were in cycle.

Supplemental Figure 3. B cell counts over time.

Every single symbol represents an APDS2 patient. Solid line: lower reference value.

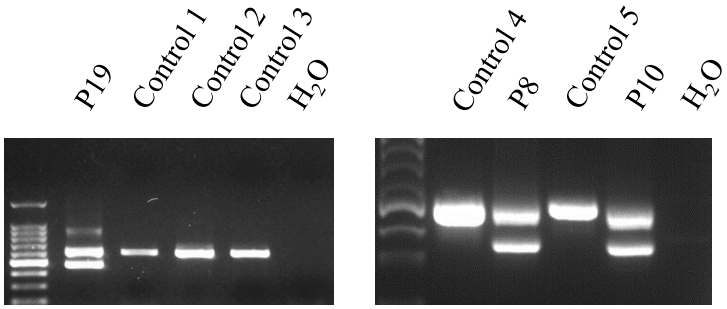
Supplemental Figure 4. CD3 T cell counts.

T cell counts before onset of any therapies**.** Solid line: lower reference value; dashed line: upper reference value.

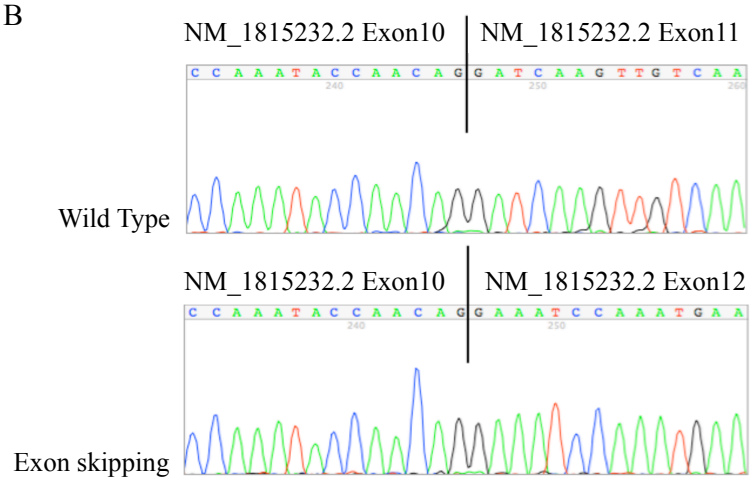
Supplemental Figure 5. Development of naive T cell numbers over time.

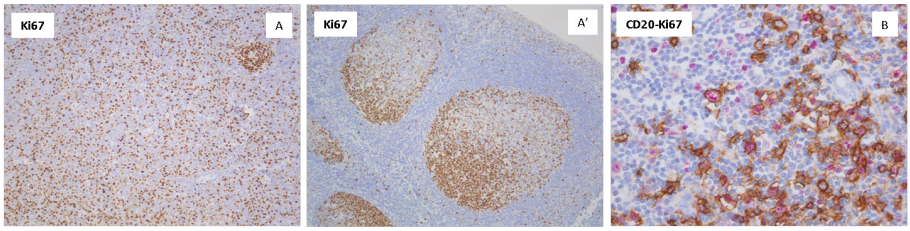
Numbers of naive CD4 (A) and naive CD8 (B) T cells. Every single symbol represents an APDS2 patient. Solid line: lower reference value.

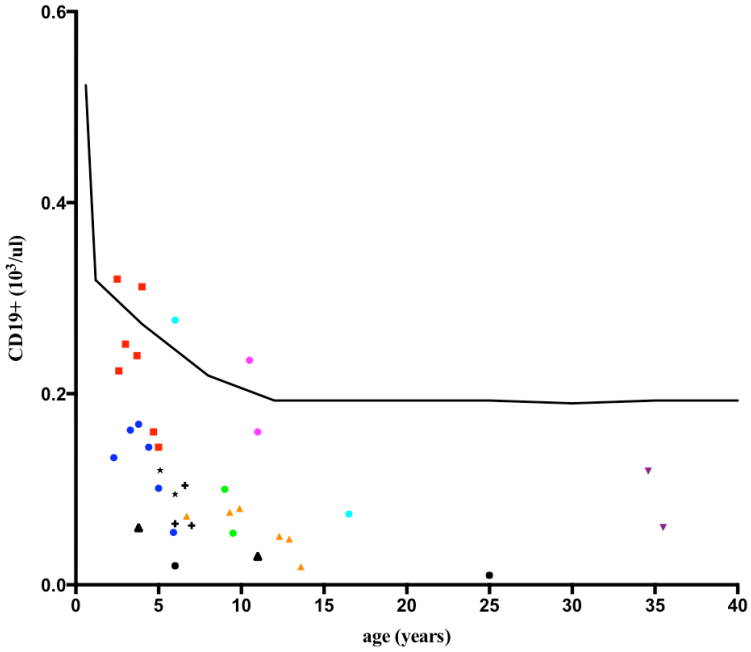
A

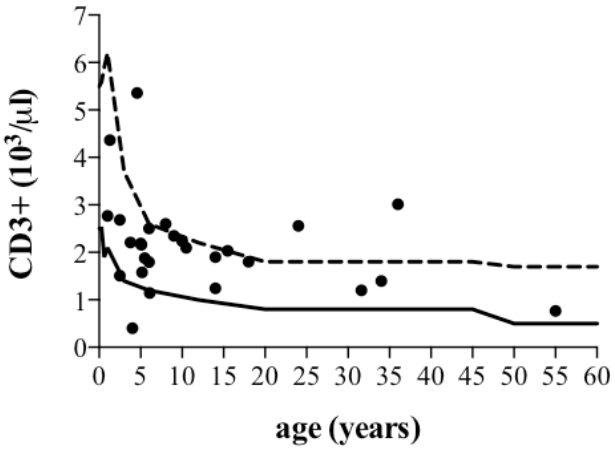

P19
Control 1
Control 2
Control 3
H2O
Control 4
P8
Control 5
P10
H2O

B

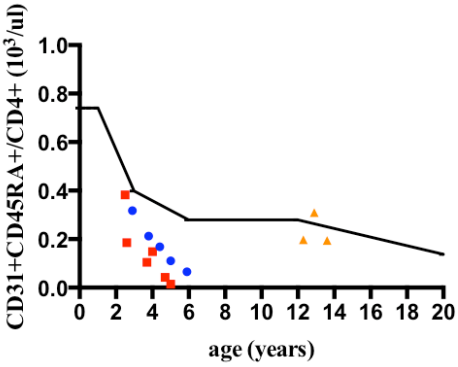

NM_1815232.2 Exon10
NM_1815232.2 Exon11
Wild Type
NM_1815232.2 Exon10
NM_1815232.2 Exon12
Exon skipping



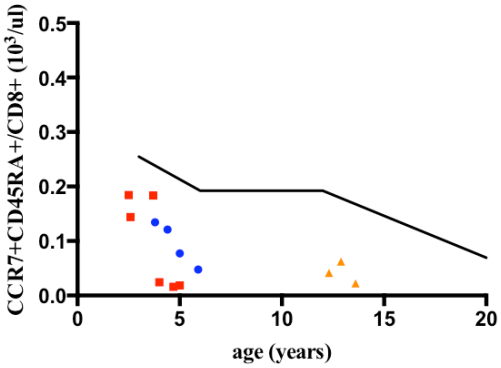

0.6
0.4
0.2
0.0
CD19+ (10³/ul)
0
5
10
15
20
25
30
35
40
age (years)


CD3+ (10^3/µl)
0
1
2
3
4
5
6
7
0 5 10 15 20 25 30 35 40 45 50 55 60
age (years)

A


CD31+CD45RA+/CD4+ (10³/ul)
1.0
0.8
0.6
0.4
0.2
0.0
0 2 4 6 8 10 12 14 16 18 20
age (years)

B


CCR7+CD45RA+/CD8+ (10³/ul)
0.5
0.4
0.3
0.2
0.1
0.0
0 5 10 15 20
age (years)