Mutations in the adaptor-binding domain and associated linker region of p110δ cause Activated PI3K-δ Syndrome 1 (APDS1)

Class I phosphoinositide-3-kinases (PI3Ks) convert phosphoinositol-4,5-bisphosphate to phosphoinositol-3,4,5-trisphosphate, a lipid second messenger produced mainly at plasma membranes where it recruits and activates pleckstrin homology domain-containing proteins, including the well known protein kinase AKT. Class IA PI3Ks are heterodimers composed of a catalytic subunit (p110α, p110β or p110δ) and a regulatory subunit (p85α, p85β or p85γ, p50α and p55α). The catalytic subunits consist of an adaptor-binding domain (ABD), a Ras-binding domain (RBD), a protein kinase C homology-2 (C2) domain, a helical domain and a kinase domain. All of the class IA regulatory subunits contain two Src homology 2 domains, nSH2 and cSH2, separated by a coiled-coil domain known as inter-SH2 domain (iSH2).[1] The p110δ catalytic subunit encoded by the *PIK3CD* gene is predominantly expressed in leukocytes. So far, six heterozygous germline gain-of-function mutations affecting either C2, helical or kinase domains of p110δ have been described to be responsible for the autosomal dominant "Activated PI3K-δ Syndrome 1" (APDS1): N334K, C416R, E525K, E525A, E1021K and E1025G[2-6] (Figure 1A, B). A clinically and biologically similar disease "Activated PI3K-δ Syndrome 2" (APDS2) is caused by mutations in the *PIK3R1* gene encoding the regulatory subunit p85α, p55α and p50α: splice site mutations responsible for amino acid 434-475 deletion.[7,8] Both diseases - although heterogeneous - share a common phenotype characterized mainly by recurrent respiratory tract infections since childhood, bronchiectasis, lymphoproliferative disorder, and predisposition to development of B-cell lymphoma as main clinical complications.[9] Biologically, APDS patients present with hypogammaglobulinemia and B-cell lymphopenia with an increased percentage of transitional B cells and decreased naive T-cell counts, especially T CD4+ cells. Here, we report three unrelated patients with novel heterozygous mutations in *PIK3CD* (E81K and G124D) located in the ABD and the ABD-RBD linker region of p110δ as cause for APDS1. These two gain-of-function mutations thus affect domains not previously shown to be important in the increased p110δ activity characteristic of this syndrome.

The patients were born at term and presented with recurrent upper and lower respiratory tract infections since childhood. For patient 1 (P1), a 13-year-old boy, no relevant family history was reported. He presented with hypogammaglobulinemia with decreased IgG and IgA but normal IgM serum levels (Table 1). Current complications include bronchiectasis, a lymphoproliferative syndrome with splenomegaly and hepatic fibrosis responsible for portal hypertension associated with gastrointestinal bleedings. P2, a 10-year-old boy, presented with recurrent otitis media and sinusitis since his first year of life. He had an adenoidectomy at 3 years of age. He presented with high IgM but normal IgG and IgA serum levels (Table 1). The serum levels of IgG2 (0.23; N: 0.56) and IgG4 (<0.002; N: 0.018) subclasses were low. P3, a 9-year-old girl, presented with growth retardation since 6 months of age (currently -

Table 1. Immunological features.

	P1 (4 years)	P2 (4 years)	P3 (4 years)	control values (4 years)	P1 (10 years)	P2 (9 years)	P3 (9 years)	control values (9-10 years)
T cells /μl (CD3+)	1248	1438	2429	1400-3700	900	1488	1887	1200-2600
CD4+ T cells /μl	496	643	749	700-2200	280	474	536	650-1500
CD8+ T cells /μl	720	713	1584	490-1300	530	948	1308	370-1100
Naïve CD4+ T cells (%) (CD31+CD45RA+/CD4+)	24	ND	ND	43-55	14	7	8	43-55
Naïve CD8+ T cells (%) (CCR7+CD45RA+/CD8+)	ND	ND	ND	68-96	20	8	3	52-68
Effector memory CD8+ T cells (%) (CCR7-CD45RA-/CD8+)	ND	ND	ND		3	48	71	11-20
Central memory CD8+ T cells (%) (CCR7+CD45RA-/CD8+)	ND	ND	ND		75	19	7	3-4
Terminal effector memory CD8+ T cells (%) (CCR7-CD45RA+/CD8+)	ND	ND	ND		1	25	19	16-28
B cells /μl (CD19+)	160	390	429	390-1400	30	98	86	270-860
Memory B cells (%) (CD27+/CD19+)	2	ND	ND	>10	NI	13	NI	14.7-25.8
Transitonal B cells (%) (CD24++CD21+/CD19+)	ND	ND	ND		13#	ND	25	2-11
Natural killer cells/μl (CD16+CD56+)	ND	113	419	200-600	70	49	86	100-480
Total IgG, g/l	2.9	9.26	11.2	4.8-9	7.6*	12.3*	16.4*	6.2-11.5
IgA, g/l	0.23	1.04	0.34	0.41-1.41	0.03	1.04	0.84	0.5-1.7
IgM, g/l	1.51	2.25	1.46	0.54-1.53	2.24	1.42	0.86	0.55-1.55

ND: Not done; NI: Not interpretable; *IgG replacement therapy; #: 13 years old at evaluation.

3 SD of height and -2.5 SD of weight). Lymphadenopathy and splenomegaly were noted at the age of 8 years, and adenoidectomy and tonsillectomy were performed. She had decreased IgA but normal IgG and IgM serum levels. Her mother had a similar clinical phenotype and died from Hodgkin lymphoma. Unfortunately, no genetic analysis of the mother could be performed since no DNA was available. All three patients received IgG replacement therapy. A CD4$^+$ T-cell lymphopenia affecting especially the naïve compartment was noticed in all three cases. In P1, an elevated number of CD8$^+$CD57$^+$ senescent T cells was found (Figure 1C). In addition, the three patients developed a progressive B-cell lymphopenia with decreased counts of memory B cells and increased percentage of transitional B

cells (Table 1 and Figure 1C). Overall, the clinical and biological phenotype of the three patients was compatible with APDS. Thus, mutations known to cause APDS1 or APDS2 were searched and excluded by Sanger sequencing. Subsequently, to identify the genetic cause of the disease, whole exome sequencing was performed in P1. A heterozygous G to A mutation at position 9775698 (GRCh37; NM_005026.3) on chromosome 1, c.241 G>A in the PIK3CD gene was detected. Neither our in-house variant database nor the Exome Sequencing Project and Exome Aggregation Consortium databases contained a record of a nucleotide variation at this position. In the Catalogue of Somatic Mutation in Cancer database, the nucleotide variation was reported as somatic mutation in a lymphoid
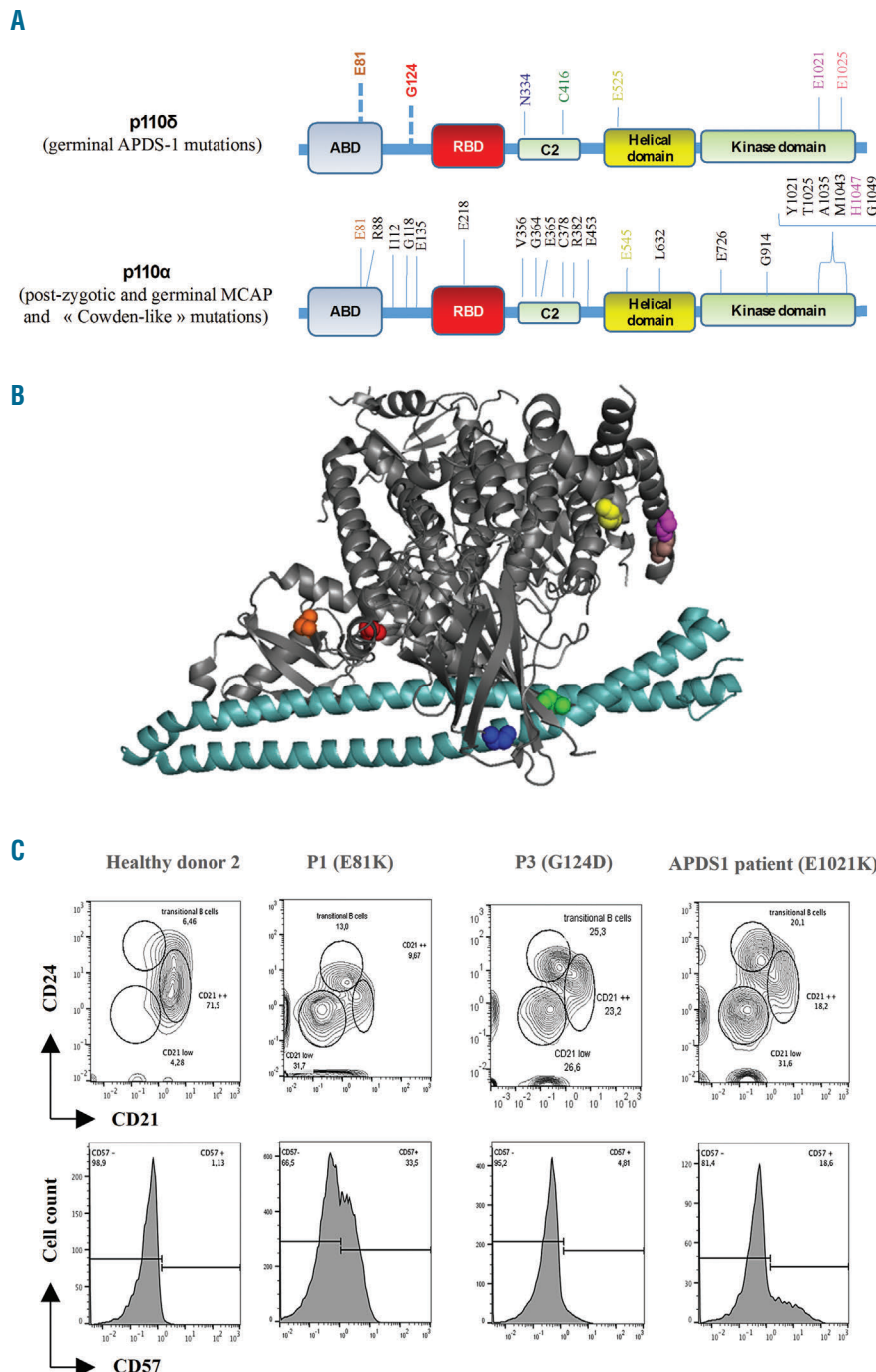

Figure 1. Localization of gain-of-function mutations in p110δ and phenotypic profile of lymphocytes. (A) Schematic representation of p110δ and p110α proteins, APDS1, MCAP and Cowden-like syndrome mutations are indicated, respectively.[2-5,14] (B) Crystal structure of p110δ and the p85α iSH2 domain (light blue); PDB ID: 5DXU,[15] the amino acids E81 (orange), G124 (red), N334 (blue), C416 (green), E525 (yellow), E1021 (magenta), and E1025 (salmon) in p110 δ are highlighted. (C) FACS plots of B cells (up) gated on CD19$^+$ and T cells (down) gated on CD3$^+$CD8$^+$. Staining was performed on total blood from P1 and P3, a healthy donor and a known APDS1 patient.

neoplasm and in a lung carcinoma. The variation encodes an amino acid substitution from glutamic acid to lysine at position 81 (E81K) located in the ABD at the N-terminal part of p110δ (Figure 1A). Sanger sequencing confirmed the mutation in the patient, which was not present in either parents. The *de novo* mutation was predicted to be highly damaging and deleterious by *in silico* prediction algorithms PolyPhen (0.99) and SIFT (0.01), respectively.

For P2 and P3, a targeted Next-Generation sequencing of a primary immunodeficiencies gene panel including *PIK3CD* and *PIK3R1* was performed. A heterozygous G to A mutation at position 9775907 (GRCh37; NM_005026.3) in the *PIK3CD* gene was detected. The mutation was not found in our in-house variant database, and was predicted to be highly damaging in PolyPhen (1.00). The mutation leads to an amino acid substitution from glycine to aspartic acid at position 124 (G124D) located in the linker region between the ABD and the RBD at the N-terminal of p110δ (Figure 1A).

To investigate whether the E81K and G124D mutations lead to a gain-of-function of p110δ activity, phosphorylation levels of AKT, a downstream protein of the PI3Kδ-sig-

nalling pathway, were determined in patients' T-cell blasts. Higher levels of phosphorylated AKT at Ser473 were observed both basally and on stimulation with OKT3 in T-cell blasts derived from P1 an P2 *versus* healthy control T-cell blasts (Figure 2A). Treatment with a p110δ specific inhibitor (IC87114) abrogated those differences, indicating that PI3Kδ-signalling was responsible for the high level of AKT phosphorylation at Ser473. In addition, phosphorylation of S6, another downstream protein of the PI3K/AKT signalling pathway, was evaluated *ex vivo* in total blood lymphocytes. B-lymphocytes from P1 and P3 consistently presented with elevated phosphorylation at Ser235/236 of S6 compared to control samples. In contrast, phosphorylation at Ser235/236 of S6 in T lymphocytes was variable as only elevated in P1 (Figure 2B). Together, our functional analysis demonstrated that the *de novo* E81K mutation located in ABD and the G124D mutation located in the ABD-RBD linker region of p110δ are gain-of-function mutations. The difference in phosphorylation at Ser235/236 of S6 level *ex vivo* in patient T lymphocytes, together with the difference in the frequency of CD8^{+}CD57^{+} T cells, further reflect the variability described
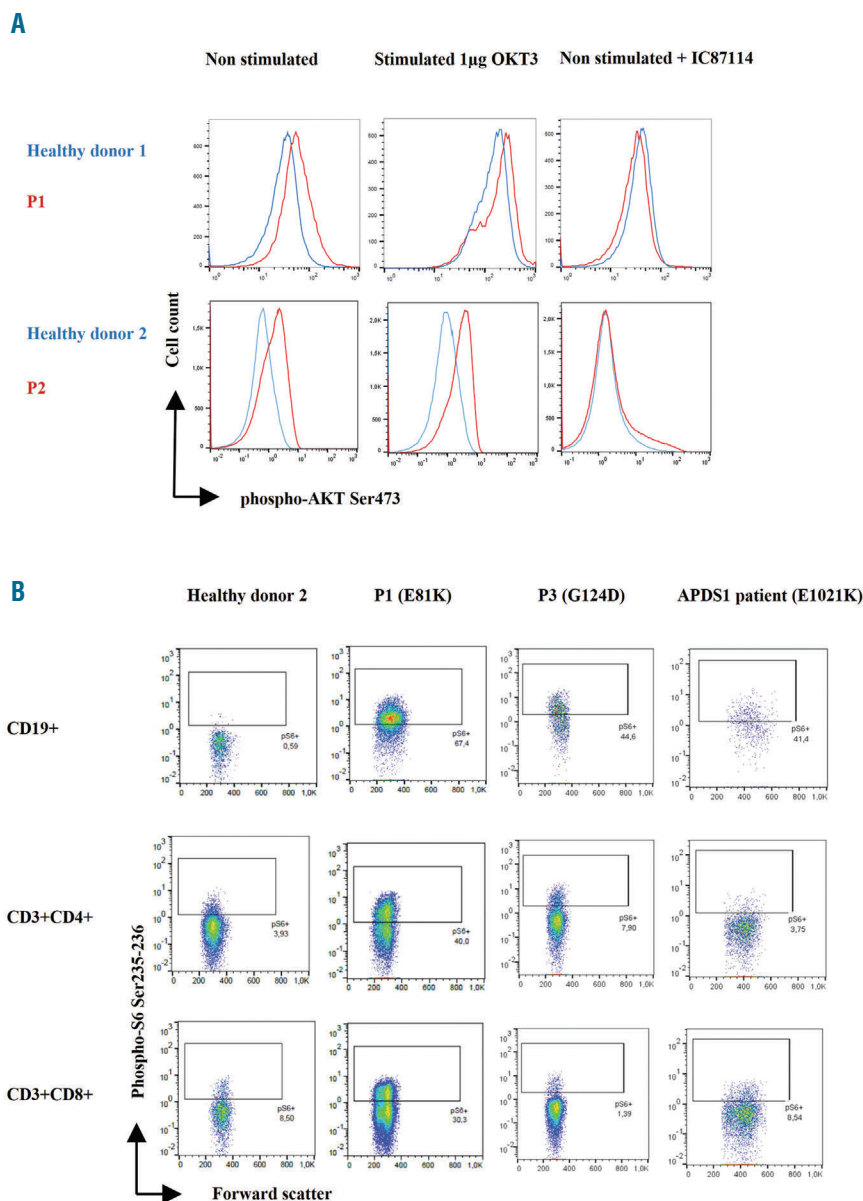

Figure 2. AKT and S6 phosphorylation study. (A) Intracellular staining of phospho-AKT Ser473 on T-cell blast (day 7 of IL2 culture) from patient and healthy donor, without and with stimulation with OKT3 or p110δ inhibitor IC87114 for 10 minutes. (B) *Ex vivo* phospho-S6 Ser235-236 intracellular staining performed on total blood from P1 and P3, a healthy donor and a known APDS1 patient.

in APDS patient cohorts,[2,10,11] possibly explained by infection histories, and environmental and genetic factors.

All of the class IA p110 isoforms are inhibited by the presence of the iSH2 and the nSH2 domains of p85. This effect is mediated by contacts between the iSH2 and C2, and contacts of the nSH2 with the C2, helical and kinase domains.[1] The published gain-of-function mutations of p110δ responsible for APDS1 map to these regulatory interfaces (Figure 1B). It has been reported that the iSH2 domain of the regulatory subunit interacts tightly with the ABD of the catalytic subunit.[12] The E81K mutation located in ABD and the G214D mutation located in its linker domain thus likely activates p110δ by mimicking or enhancing specific conformational changes that are required for the PI3Kδ catalytic activity. The class IA catalytic subunits p110α (encoded by *PIK3CA*) and p110δ share a 72% amino acid homology.[3] Intriguingly, gain-of-function mutations in *PIK3CA* at positions corresponding to mutations observed in *PIK3CD* causing APDS1 (including the E81K and G124D mutations) have been described in cancers, and some of them are responsible for the megalencephaly-capillary malformation-polymicrogyria syndrome (MCAP)[13,14] (Figure 1A). This syndrome is characterized by overgrowth, brain and body asymmetry, cutaneous vascular malformations, digital anomalies, connective tissue dysplasia in joints, cortical brain malformations, and polymicrogyria. Thus, gain-of-function mutations in *PIK3CD* and *PIK3CA* responsible for APDS1 and MCAP, respectively, are likely to share similar activation mechanisms. Of note, mutations in the regulatory subunits encoded by *PIK3R1* and *PIK3R2* are responsible for APDS2 and MCAP, respectively.[7,13]

Precise genetic diagnosis of APDS is essential for patients to allow better prognostic measures and adequate treatment and follow up. Successful allogeneic hematopoietic stem cell transplantations (HSCT) as treatment for APDS were reported for severe cases.[11] APDS patients presenting with signs of lymphoproliferation were started from their diagnosis on long-term rapamycin treatment, which appears efficient in most cases on clinical manifestations.[3,11,10] Since the diagnosis of APDS1, P1 and P3 were treated with rapamycin and are under consideration for HSCT. Inhibitors specific for p110δ are currently in clinical trials (EudraCT Numbers: 2015-002900-10; 2015-005541-30; 2014-003876-22; 2015-004876-31) and could offer a new treatment option for APDS patients with possibly higher efficiency and less unwanted side effects.

Finally, our work describing two new *PIK3CD* gain-of-function mutations, E81K and G124D, demonstrated that mutations in the ABD and its linker region lead to APDS1. It highlights that mutations occurring in different parts of the gene can lead to the very same consequences, and should thus be screened in patients with a phenotype resembling APDS.

*Lucie Heurtier,[1,2] Hicham Lamrini,[1,2] Loïc Chentout,[1,2]
Marie-Céline Deau,[1,2] Amine Bouafia,[1,2] Jérémie Rosain,[3]
Jean-Marc Plaza,[4] Mélanie Parisot,[5] Benoit Dumont,[6]
Delphine Turpin,[7] Etienne Merlin,[8] Despina Moshous,[1,2,9]
Nathalie Aladjidi,[10] Bénédicte Neven,[1,2,9] Capucine Picard,[1,2,3]
Marina Cavazzana,[1,2,11] Alain Fischer,[1,2,9,12] Anne Durandy,[1,2]
Jean-Louis Stephan[6] and Sven Kracker[1,2]*

[1]Université Paris Descartes, Sorbonne Paris Cité, Imagine Institute; [2]INSERM UMR 1163, Imagine Institute, Paris; [3]Study Center for Primary Immunodeficiencies, Assistance Publique Hôpitaux de Paris (AP-HP), Necker Children Hospital; [4]Bioinformatic Platform, Université Paris Descartes, Sorbonne Paris Cité, Imagine Institute; [5]Genomic Core Facility, Université Paris Descartes, Sorbonne Paris Cité, Imagine Institute; [6]Department of Pediatrics, Hôpital Nord,

Saint-Etienne; [7]Laboratory of Immunology and Immunogenetics, Bordeaux University Hospital; [8]Department of multidisciplinary Pediatrics, CHU d'Estaing, Clermont-Ferrand; [9]Department of Pediatric Immunology, Hematology and Rheumatology, Assistance Publique-Hôpitaux de Paris (AP-HP), Necker Children Hospital; [10]Pediatric Hematologic Unit /CIC 1401, Inserm CICP, University Hospital of Bordeaux, Pediatric Hospital; [11]Biotherapy Department, Centre d'Investigation Clinique intégré en Biothérapies, Necker Children Hospital, Assistance Publique-Hôpitaux de Paris (AP-HP), and [12]Collège de France, Paris, France

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