# Novel heterozygous PIK3CD mutation presenting with only laboratory markers of combined immunodeficiency

Amarilla B. Mandola[a,b], Harjit Dadi[a,b], Brenda Reid[a,b], and Chaim M. Roifman[a,b]*




## ABSTRACT

**Introduction:** Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta (PIK3CD) is one part of a heterodimer forming the enzyme phosphoinositide 3-kinase (PI3K), found primarily in leukocytes. PIK3CD generates phosphatidyl-inositol 3,4,5-trisphosphate (PIP3), and is involved in cell growth, survival, proliferation, motility, and morphology. An increasing number of patients have been described with heterozygous PIK3CD gain-of-function (GOF) mutations, leading to combined immunodeficiency with both B- and T-cell dysfunction. Patients suffer recurrent respiratory infections, often associated with bronchiectasis and ear and sinus damage, as well as severe recurrent or persistent infections by herpesviruses, including EBV-induced lymphoproliferation.

**Aim:** To present the clinical phenotypic variability of a novel PI3KCD mutation within a family.

**Methods:** Patient information was collected prospectively and retrospectively from medical records. Comprehensive immune work up, genetic, and signaling evaluation was performed.

**Results:** We describe here 2 patients, daughter and mother, with heterozygous PIK3CD mutation identified by whole exome sequencing and Sanger confirmation. The child was screen-positive by newborn screening for severe combined immunodeficiency (SCID). Cellular assays revealed an increase in the baseline phosphorylation of T cells in the patient. Furthermore, both patients had hyper-activation of the catalytic domain, resulting in increased phosphorylation of AKT upon activation.

**Discussion:** GOF mutations affecting the PIK3CD gene are associated with an increased risk for lymphoproliferation leading to Activated PIK3-delta syndrome (APDS). The clinical course of APDS is highly variable, ranging from combined immunodeficiency with recurrent infections, autoimmune complications, and requiring stem cell transplantation, through isolated antibody deficiency, to asymptomatic adults. Our patient is the first to be identified by newborn screening for SCID. Surprisingly, the clinical course has so far been unremarkable, as well, the mother appears to be completely asymptomatic. Nevertheless, the persistent lymphopenia indicates PIK3CD dysfunction. Because of the wide gap between laboratory findings and clinical manifestations, this kindred poses both a diagnostic as well treatment challenge.

**Statement of novelty:** We report here a novel PIK3CD mutation diagnosed due to abnormal newborn screen for SCID.


[a]Division of Immunology and Allergy, Department of Pediatrics, The Hospital for Sick Children and the University of Toronto, Toronto, ON; [b]The Canadian Centre for Primary Immunodeficiency and The Jeffrey Modell Research Laboratory for the Diagnosis of Primary Immunodeficiency, The Hospital for Sick Children, Toronto, ON

*Corresponding author: Chaim M. Roifman/chaim.roifman@sickkids.ca

Submitted 10 February 2020
Accepted 13 April 2020
Available online 6 May 2020

**LymphoSign Journal 7:49–55 (2020)**
dx.doi.org/10.14785/lymphosign-2020-0003

## Introduction

Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta (PIK3CD; p110δ) together with the regulatory subunit p85, forms the lipid kinase phosphoinositide 3-kinase (PI3K) found primarily in leukocytes. This enzyme is critical for signaling downstream of multiple receptors, thus controlling cell growth, maturation, and morphology. Its primary function is to recruit Pleckstrin Homology (PH) domain-containing proteins such as AKT and PDK1 to the membrane, allowing for the activation of downstream signaling cascades.

The signaling pathways mediated by PIK3CD are essential for B cell receptor (BCR) induced responses, as well as Toll-like receptor (TLR)4 and TLR9 mediated cytokine secretion. In T cells, PIK3CD is involved in T cell receptor mediated synapse formation, proliferation, differentiation and cell migration. In NK cells, PIK3CD is required for their activation, maturation, migration and cytokine production.

Recently, an increasing number of patients with combined immunodeficiency and Epstein–Barr virus (EBV) induced lymphoproliferation were found to carry heterozygous PIK3CD gain-of-function (GOF) mutations.

## Methods

### Patients

Informed consent was obtained. This study conforms to the Declaration of Helsinki and all local ethical requirements. Information on presentation, complications, laboratory parameters, management, and outcomes were compiled both prospectively and retrospectively using parent interview and medical note review.

### Lymphocyte proliferation

Lymphocyte proliferative responses to mitogens including phytohemagglutinin (PHA) and anti-CD3 were evaluated. All assays were performed in triplicate and were compared with simultaneously stimulated normal controls, as previously described (Sharfe et al. 2014).

### Western blotting

Studies were performed on Ficoll separated peripheral blood lymphocytes as well as T cells that were sorted by flow cytometry. Surface phenotypes were determined on a Coulter EPICS V Flow Cytometer (Beckman Coulter, Brea, CA, USA). Both patient and control cells were obtained, and either left unstimulated or were stimulated with anti-CD3 UCHT1 antibody (5 μg for $4 \times 10^6$ cells) for 10 minutes at 37 °C. Cells were subsequently lysed in 1% Triton X-100 vanadate lysis buffer and protein expression assessed by Western blotting. All blots were repeated at least twice. The primary antibodies used for Western blotting were: Anti-pAKT Ser473 (Invitrogen) and anti-Gα(i) (Cell Signaling), followed by appropriate horseradish peroxidase-conjugated secondary antibody. Immunoreactive proteins were visualized by enhanced chemiluminescence.

### Whole exome sequencing and variant calling

DNA from blood was submitted to The Centre for Applied Genomics (TCAG), Toronto, ON, Canada for exome library preparation and sequencing. DNA was quantified by Qubit DNA HS assay (Life Technologies, Carlsbad, CA, USA) and 100 ng of input DNA was used for library preparation using the Ion AmpliSeq Exome Kit (Life Technologies) according to the manufacturer's recommendations. The Ampliseq Exome library was immobilized on Ion PI™ Ion Sphere™ particles using the Ion PI Template OT2 200 Kit v3. Sequencing was performed with the Ion PI Sequencing 200 Kit v3 and Ion PI Chip v2 in the Ion Proton™ semiconductor sequencing system following the manufacturer's recommendation.

Alignment and variant calling were performed using Torrent Suite (version 4.0) on the Ion Proton Server, using the Ion Proton ampliseq germline low stringency setting and the hg19 reference genome. The variants were annotated using an in-house annotation pipeline (Stavropoulos et al. 2016) based on Annovar (November 2014 version) (Wang et al. 2010) and RefSeq gene models (downloaded from UCSC 1 August 2015).

### Sanger sequencing

Patient genomic DNA was extracted from peripheral blood lymphocytes using the Geneaid Genomic DNA Mini Kit. Genomic DNA was amplified by polymerase chain reaction (PCR) with specific primers designed upstream and downstream of the PI3KCD gene. Sequencing was done using GenomeLab Dye

Terminator Cycle Sequencing (DTCS) Quick Start Kit (Beckman Coulter) and analyzed on CEQ 8000 Genetic Analysis System (Beckman Coulter).

# Results

## Patients

Patient 1 is a 3.5 year old female, born at term after a normal pregnancy to non-consanguineous parents of English descent. She was found to have markedly reduced T cell receptor excision circle (TREC) levels by newborn screening for severe combined immunodeficiency (SCID). Her mother was not treated during pregnancy with immunosuppressive drugs which may have affected T cell number and function, and the infant had an unremarkable perinatal course.

Her infectious history consisted of several upper respiratory tract infections which didn't require hospital admission. Her growth and development were normal. She followed the 25th and 50th percentile for height and weight, respectively. On physical examination she had small palpable lymph nodes bilaterally and her tonsils were visible. She had mild eczema of the cheeks and mild esotropia. On auscultation she had a 3/6 midsystolic murmur and a S2 click, likely caused by pulmonic stenosis documented on echocardiography. While she met all her developmental milestones on time, she had repeated and frequent falls which were attributed to her vision problems due to esotropia.

Patient 2, the mother of patient 1, was evaluated following the assessment of her daughter. She had a history of childhood eczema and repeated otitis but had no health issues during her adulthood.

Family history is remarkable for asthma, in both patient 1, her sister, and father. The father also had a congenital heart defect which was repaired. There is also history of type 2 Diabetes Mellitus in both maternal and paternal grandparents, and the maternal grandmother had thyroid disease.

## Immune evaluation

Patient 1's laboratory results were remarkable for intermittent leukopenia, attributed to lymphopenia and intermittent neutropenia. On lymphocyte immunophenotyping, she was found to have reduced numbers of CD3+, CD4+, and CD8+ T cells, normal NK and CD19+ B cell count (Tables 1 and 2).

***Table 1:*** **Immunolaboratory work up.**

| | Patient 1 | | | | | | Patient 2 | |
|---|---|---|---|---|---|---|---|---|
| Lab parameters | 2 mo | 6 mo | 18 mo | 20 mo | 26 mo | Reference range | 30 y | Reference range |
| WBCs ($\times 10^9$/L) | 5.1 | 3.8 | 4.9 | 4.3 | 6.15 | 4–13 | 8 | 4.37–9.6 |
| Neutrophils ($\times 10^9$/L) | **1.48** | **1.03** | **0.81** | 1.98 | 3.62 | 1.6–8 | 3.8 | 2–7.15 |
| Lymphocytes ($\times 10^9$/L) | 2.75 | 2.09 | 3.09 | 1.32 | 1.65 | 1.2–5.77 | 3.36 | 1.16–3.18 |
| Eosinophils ($\times 10^9$/L) | 0.26 | 0.19 | 0.52 | 0.47 | 0.23 | 0.03–0.46 | 0.1 | 0.03–0.27 |
| PLT ($\times 10^9$/L) | 403 | 332 | 395 | 384 | 293 | 189–394 | 287 | 186–353 |
| CD3+ (cells/μL) | **1110** | **1027** | **1399** | — | **927** | 2000–6900 | 2444 | 700–2100 |
| CD19+ (cells/μL) | 1094 | 1051 | 1242 | — | **430** | 700–2500 | 623 | 100–500 |
| CD3+/CD4+ (cells/μL) | **890** | **823** | **1022** | — | **618** | 1400–5100 | 1494 | 300–1400 |
| CD3+/CD8+ (cells/μL) | **223** | **200** | **317** | — | **269** | 600–2200 | 624 | 200–900 |
| NK (cells/μL) | 499 | 114 | 383 | — | 218 | 100–1000 | 208 | 90–600 |
| PHA SI | **374** | **197** | **388** | — | — | SI > 400 (>50% of ctrl) | 417 | SI > 400 (>50% of ctrl) |
| Anti-tetanus Ab | — | 0.12 | 0.17 | 1.17 | 0.31 | >0.1 IU/mL | 0.98 | >0.1 IU/mL |
| Diptheria Ab | — | — | — | — | 2.1 | >0.1 IU/mL | — | >0.1 IU/mL |
| MMRV Ab | NA | NA | NA | NA | NA | — | Protective | — |
| EBV, CMV, HHV6 PCR | — | — | — | — | NEG | — | — | — |
| EBV IgG (EA, EBNA, VCA) | — | — | — | — | NEG | — | +VCA, EBNA IgG | — |
| Anti A titre | — | — | 8 | — | 16 | — | — | — |

**Note:** WBC, white blood cell; SI, stimulation index; MMRV, mumps, measles, rubella and varicella. PLT, platelets; NK, natural killer cells; PHA SI, phytohemagglutinin stimulation index; EBV, epstein barr virus; CMV, cytomegalovirus; HHV6, human herpesvirus 6; EA, early antigen; EBNA, EBV nuclear antigen antibody; VCA, EBV viral capsid antigen antibody.

***Table 2:*** **Memory/naïve T cell flow cytometry.**

| | Patient 1 | | Patient 2 | |
|---|---|---|---|---|
| | Age 26 mo | | Age 30 y | |
| | Patient | Control | Patient | Control |
| CD3+/CD45+ RA+ | 55.3 | 56.2 | 41.8 | 56.2 |
| CD3+/CD45+ RO+ | 37.6 | 38.5 | 53.4 | 38.5 |
| CD4+/CD45+ RA+ | 31.6 | 31.1 | 24.3 | 31.1 |
| CD4+/CD45+ RO+ | **24.9** | 9.2 | **30.5** | 9.2 |
| CD8+/CD45+ RA+ | 16.6 | 19.1 | 14.2 | 19.1 |
| CD8+/CD45+ RO+ | 7.9 | 19.6 | 15.9 | 19.6 |

The number of CD4+ naïve T cells were consistently lower than controls suggesting ineffective thymic production or egress of these cells. T cell receptor repertoire was found normal on multiple evaluations. Her initial TREC levels were reduced to 11.3 copies/3 μL DNA which was confirmed by a low whole blood TREC of 588 copies/0.5 μg DNA (normal >700) (Table 3). TREC levels continued to be reduced at the age of 2 months (379) but subsequently rose to the normal range (786). In vitro responses to mitogens and antigens were reduced until the age 18 months. While PHA responses normalized, responses to CD3 remained reduced. Immunoglobulin levels were normal as well as antibody titres to vaccines.

Patient 2's laboratory work up showed normal lymphocyte immunophenotyping. Like her daughter, she had lower numbers of CD4+ cells, naïve cells, and elevated memory population compared to control (Tables 1 and 2). Her in vitro response to PHA was low and proliferative responses to specific antigens including tetanus, Herpes simplex and cytomegalovirus (CMV) were below control values. She had normal immunoglobulin levels, with sustained specific antibody titres to mumps, measles, rubella, varicella, and tetanus, as well as IgG antibodies to EBV. However, evaluation of EBV and CMV particles by PCR were negative.

## Genetic work up

Following the finding of a positive newborn screen for SCID, the following tests were performed: (*i*) to exclude Di George syndrome, cytogenetic evaluation using FISH showed no microdeletion at 22q11.2. G-band analysis showed no significant chromosome arrangements and no elevated frequency of spontaneous breakage. (*ii*) SCID panel for the 20 most frequent SCID-causing genes was negative. (*iii*) We proceeded to perform whole exome sequencing (WES), which revealed heterozygous mutation in PIK3CD: c.1495G>A (Figure 1), predicting the amino acid substitution p.Glu499Lys in PIK3CD. This mutation affects the PIK helical domain of the p110 subunit (Figure 2), which likely disrupts specific inhibitory interactions between p110δ and the inter-SH2 and N-terminal SH2 domains of p85α (Lucas et al. 2014).The same variant was found in the patient's mother. No other relevant variants were identified.

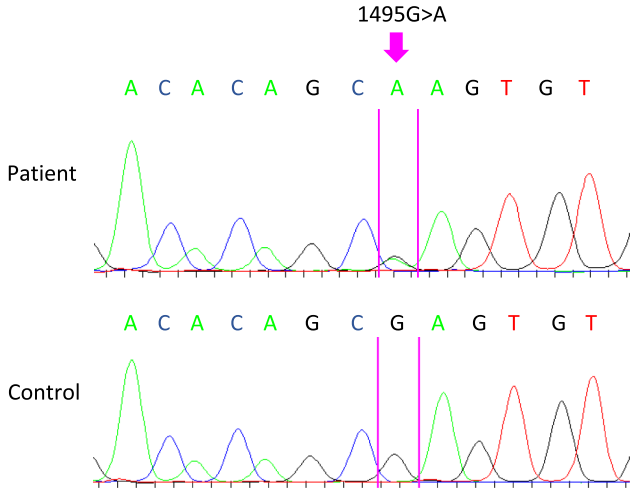


*Figure 1:* Electropherogram of the PIK3CD sequence in our patients show a heterozygous novel mutation, c.1495G>A, resulting in amino acid change p.Glu499Lys.

## Cell signaling

Western blot of anti-CD3 stimulated post-Ficoll peripheral blood lymphocytes derived from patients or control cells showed a clear increase in pAKT in both unstimulated and stimulated cells of patient 1 (Figure 3A). Increased AKT phosphorylation was also confirmed in both patients using purified T cells

***Table 3:*** **TREC results of Patient 1.**

| | Birth | 2 mo | 10 mo | 18 mo | Cut off[a] |
|---|---|---|---|---|---|
| NBS TRECs (copies/3 μL DNA) | **11.3** | — | — | — | 75/3 μL |
| WB TRECs (copies/0.5 μg DNA) | **588** | **379** | 786 | 1057 | 700/0.5 μg |

**Note:** NBS, newborn screening; TRECs, T cell receptor excision circles; WB, whole blood.
[a]Roifman et al. 2012.

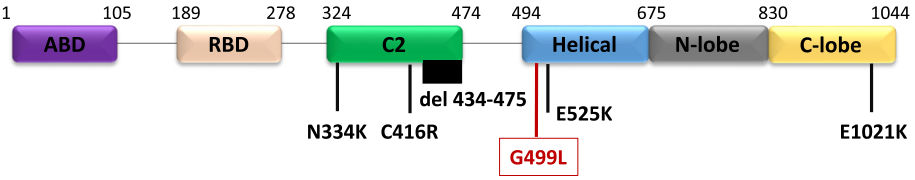


*Figure 2:* PIK3CD protein domains and mutations. Previously reported mutations are shown in black. The most common, E1021K, localized to the C-lobe of the kinase domain leading to increased lipid kinase activity, was described only in symptomatic patients and not healthy kindreds. The mutation found in our patients, G499L, affects the helical domain.

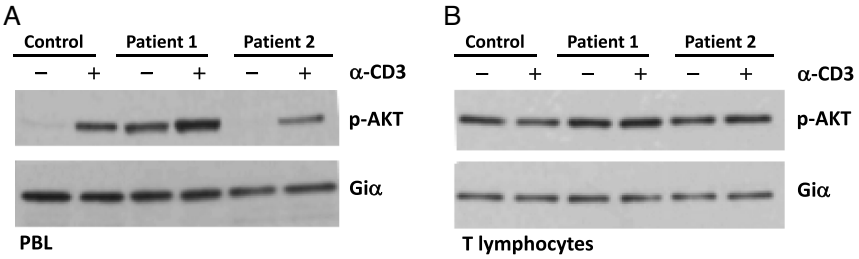


*Figure 3:* Expression of p-AKT in peripheral blood lymphocytes and T lymphocytes. Western blot analysis revealed an increase in p-AKT in both unstimulated and anti-CD3 (UCHT1) stimulated PBL (A) and T lymphocytes (B) of patient 1. Blots were stained with Giα to confirm equal loading of cell lysates.

stimulated with anti-CD3 antibody (Figure 3B). Even basal level of AKT phosphorylation were increased in patient T cells. Together, these clearly demonstrated exaggerated PIK3CD activity.

## Discussion

GOF mutations affecting the PIK3CD gene, encoding the catalytic subunit of PI3K, have been described in the past few years in a growing number of patients who present with recurring respiratory infections. Invariably, the GOF nature of the defect is detected by demonstrating increase and exaggerated activity of the PIK3CD downstream signaling pathway (Figure 4), such as AKT activation. Indeed, our patients were found to have increased AKT phosphorylation consistent with the diagnosis of activated PIK3CD syndrome (APDS).

The impact of this GOF mutation on the immune system encompasses various degrees of humoral as well as T cell dysfunction. Most commonly, CD4+ lymphopenia as well as a reduction in the number of circulating CD4+ naïve cells indicating some maturational defect in T cells. Both were encountered in our patients. Another frequently observed abnormality is low serum IgG with or without IgM levels (Coulter et al. 2017), with variable ability to respond appropriately to vaccinations (Elgizouli et al. 2016). Consistent with these observations, our patients were found to have somewhat elevated IgM levels.

The clinical manifestations can be variable even within families who carry the same mutation. Most patients present with recurrent sinopulmonary infections (Coulter et al. 2017). Susceptibility to herpesvirus infections with resultant lymphoproliferation, whether benign or malignant (lymphoma), are also prevalent. Similarly frequent are associated autoimmune disorders such as immune cytopenias, colitis, sclerosing cholangitis and others (Crank et al. 2014; Hartman et al. 2015; Elgizouli et al. 2016; Coulter et al. 2017).

For clinically symptomatic patients with APDS, especially those who experience lymphoproliferation, new selective therapeutic options have been recently offered. These include the specific inhibitors of PIK3CD: Idelalisib, which is already widely available, and leniolisib, that is still investigational. Both drugs seem to suppress PIK3CD signaling and are able to correct immune aberrations (Dornan et al. 2017; Greenwell et al. 2017; Rao et al. 2017). However, their use is

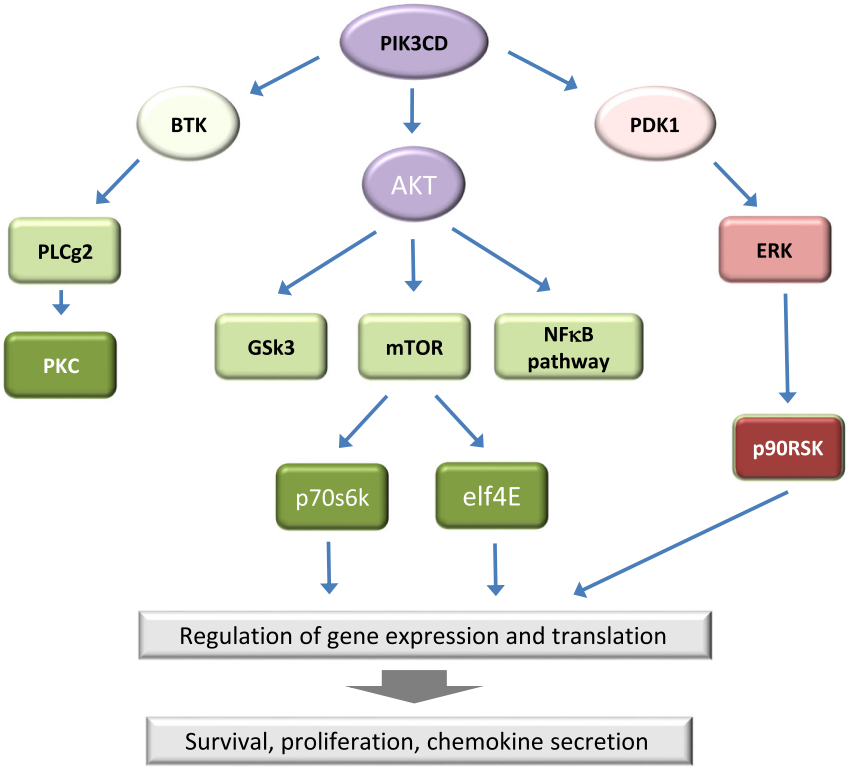


*Figure 4:* Schematic diagram of signaling cascade downstream of PIK3CD.

currently restricted to malignancies and severe clinical manifestations, clearly not relevant to our patients who are essentially asymptomatic. It is possible that early correction of PIK3CD mediated exaggerated signaling could prevent future development of complications such as lymphoproliferation and lymphoma, but so far this possibility has not been studied.

We have shown here for the first time that APDS can be detected early by TREC-based newborn screening. This intuitively would have predicted a more severe cause of this disease. By contrast, no significant clinical manifestations occurred so far in patient 1 nor her mother, who has carried the identical mutation for 3 decades. Nevertheless, the appearance of symptoms at an older age cannot be excluded. These cases may indicate that lymphoproliferation and lymphoma appearing in adults, even though they have been asymptomatic throughout their lives, could be potentially caused by a GOF mutation in PIK3CD.

## Conclusion

We have demonstrated here that APDS can be detected early by newborn screening for SCID. In spite of the molecular and immune aberrations typical of the disorder, the patients (daughter and mother) showed no clinical manifestations. This report highlights the great variability observed in APDS.

## REFERENCES

Coulter, T.I., Chandra, A., Bacon, C.M., Babar, J., Curtis, J., Screaton, N., Goodlad, J.R., Farmer, G., Steele, C.L., Leahy, T.R., Doffinger, R., Baxendale, H., Bernatoniene, J., Edgar, J.D., Longhurst, H.J., Ehl, S., Speckmann, C., Grimbacher, B., Sediva, A., Milota, T., Faust, S.N., Williams, A.P., Hayman, G., Kucuk, Z.Y., Hague, R., French, P., Brooker, R., Forsyth, P., Herriot, R., Cancrini, C., Palma, P., Ariganello, P., Conlon, N., Feighery, C., Gavin, P.J., Jones, A., Imai, K., Ibrahim, M.A., Markelj, G., Abinun, M., Rieux-Laucat, F., Latour, S., Pellier, I., Fischer, A., Touzot, F., Casanova, J.L., Durandy, A., Burns, S.O., Savic, S., Kumararatne, D.S., Moshous, D., Kracker, S., Vanhaesebroeck, B., Okkenhaug, K., Picard, C., Nejentsev, S., Condliffe, A.M., and Cant, A.J. 2017. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: A large patient cohort study. J. Allergy Clin. Immunol. **139**: 597–606.e4. PMID: 27555459. doi: 10.1016/j.jaci.2016.06.021.

Crank, M.C., Grossman, J.K., Moir, S., Pittaluga, S., Buckner, C.M., Kardava, L., Agharahimi, A., Meuwissen, H., Stoddard, J., Niemela, J., Kuehn, H., and Rosenzweig, S.D. 2014. Mutations in *PIK3CD* can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. J. Clin. Immunol. **34**: 272–276. PMID: 24610295. doi: 10.1007/s10875-014-0012-9.

Dornan, G.L., Siempelkamp, B.D., Jenkins, M.L., Vadas, O., Lucas, C.L., and Burke, J.E. 2017. Conformational disruption of PI3Kδ regulation by immunodeficiency mutations in *PIK3CD* and *PIK3R1*. Proc. Natl. Acad. Sci. U.S.A. **114**: 1982–1987. PMID: 28167755. doi: 10.1073/pnas.1617244114.

Elgizouli, M., Lowe, D.M., Speckmann, C., Schubert, D., Hulsdunker, J., Eskandarian, Z., Dudek, A., Schmitt-Graeff, A., Wanders, J., Jorgensen, S.F., Fevang, B., Salzer, U., Nieters, A., Burns, S., and Grimbacher, B. 2016. Activating PI3Kδ mutations in a cohort of 669 patients with primary immunodeficiency. Clin. Exp. Immunol. **183**: 221–229. PMID: 26437962. doi: 10.1111/cei.12706.

Greenwell, I.B., Ip, A., and Cohen, J.B. 2017. PI3K inhibitors: Understanding toxicity mechanisms and management. Oncology, **31**: 821–828. PMID: 29179250.

Hartman, H.N., Niemela, J., Hintermeyer, M.K., Garofalo, M., Stoddard, J., Verbsky, J.W., Rosenzweig, S.D., and Routes, J.M. 2015. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. J. Clin. Immunol. **35**: 11–14. PMID: 25352054. doi: 10.1007/s10875-014-0109-1.

Lucas, C.L., Kuehn, H.S., Zhao, F., Niemela, J.E., Deenick, E.K., Palendira, U., Avery, D.T., Moens, L., Cannons, J.L., Biancalana, M., Stoddard, J., Ouyang, W., Frucht, D.M., Rao, V.K., Atkinson, T.P., Agharahimi, A., Hussey, A.A., Folio, L.R., Olivier, K.N., Fleisher, T.A., Pittaluga, S., Holland, S.M., Cohen, J.I., Oliveira, J.B., Tangye, S.G., Schwartzberg, P.L., Lenardo, M.J., and Uzel, G. 2014. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. Nat. Immunol. **15**: 88–97. PMID: 24165795. doi: 10.1038/ni.2771.

Rao, V.K., Webster, S., Dalm, V.A.S.H., Šedivá, A., van Hagen, P.M., Holland, S., Rosenzweig, S.D., Christ, A.D., Sloth, B., Cabanski, M., Joshi, A.D., de Buck, S., Doucet, J., Guerini, D., Kalis, C., Pylvaenaeinen, I., Soldermann, N., Kashyap, A., Uzel, G., Lenardo, M.J., Patel, D.D., Lucas, C.L., and Burkhart, C. 2017. Effective "activated PI3Kδ syndrome"-targeted therapy with the PI3Kδ inhibitor leniolisib. Blood, **130**: 2307–2316. PMID: 28972011. doi: 10.1182/blood-2017-08-801191.

Roifman, C.M., Somech, R., Kavadas, F., Pires, L., Nahum, A., Dalal, I., and Grunebaum, E. 2012. Defining combined immunodeficiency. J. Allergy Clin. Immunol. **130**: 177–183. PMID: 22664165. doi: 10.1016/j.jaci.2012.04.029.

Sharfe, N., Nahum, A., Newell, A., Dadi, H., Ngan, B., Pereira, S.L., Herbrick, J.A., and Roifman, C.M. 2014. Fatal combined immunodeficiency associated with heterozygous mutation in *STAT1*. J. Allergy Clin. Immunol. **133**: 807–817. PMID: 24239102. doi: 10.1016/j.jaci.2013.09.032.

Stavropoulos, D.J., Merico, D., Jobling, R., Bowdin, S., Monfared, N., Thiruvahindrapuram, B., Nalpathamkalam, T., Pellecchia, G., Yuen, R.K.C., Szego, M.J., Hayeems, R.Z., Shaul, R.Z., Brudno, M., Girdea, M., Frey, B., Alipanahi, B., Ahmed, S., Babul-Hirji, R., Porras, R.B., Carter, M.T., Chad, L., Chaudhry, A., Chitayat, D., Doust, S.J., Cytrynbaum, C., Dupuis, L., Ejaz, R., Fishman, L., Guerin, A., Hashemi, B., Helal, M., Hewson, S., Inbar-Feigenberg, M., Kannu, P., Karp, N., Kim, R., Kronick, J., Liston, E., MacDonald, H., Mercimek-Mahmutoglu, S., Mendoza-Londono, R., Nasr, E., Nimmo, G., Parkinson, N., Quercia, N., Raiman, J., Roifman, M., Schulze, A., Shugar, A., Shuman, C., Sinajon, P., Siriwardena, K., Weksberg, R., Yoon, G., Carew, C., Erickson, R., Leach, R.A., Klein, R., Ray, P.N., Meyn, M.S., Scherer, S.W., Cohn, R.D., and Marshall, C.R. 2016. Whole genome sequencing expands diagnostic utility and improves clinical management in pediatric medicine. NPJ Genom Med. **1**: 15012. PMID: 28567303. doi: 10.1038/npjgenmed.2015.12.

Wang, K., Li, M., and Hakonarson, H. 2010. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. **38**: e164. PMID: 20601685. doi: 10.1093/nar/gkq603.