Four diseases, PLAID, APLAID, FCAS3 and CVID and one gene (PHOSPHOLIPASE C, GAMMA-2; PLCG2) : striking clinical phenotypic overlap and difference

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Abstract

We suggest PLAID,APLAID and FCAS3 have to be considered as same diseases,because of our long-term clinical experiences and genetic results in six patients.Small proportion of CVID patients are also PLAID/APLAID/FCAS3 patients and all these have disease-causing-mutations in PLCG2-genes,so it may be better to define all of them as "PLCG2 deficiency".

Key Clinical Message: Germline mutations in PLCG2 gene cause PLAID,APLAID,FCAS3, and CVID.Clinical experiences in patients with PLCG2 mutations led us to consider that PLAID, APLAID and FCAS3 are same diseases.It may be better to define all of them as "PLCG2 deficiency".

INTRODUCTION

The PLCG2 gene which is located on the 16th chromosome (16q23.3) encodes phospholipase Cγ2 (PLCG2), a transmembrane signaling enzyme that catalyzes the production of second messenger molecules utilizing calcium as a cofactor and propagates downstream signals in several hematopoietic cells (1). Recently, heterozygous germline mutations in human PLCG2 were linked to some clinical phenotypes with some overlapping features—PLCγ2-associated antibody deficiency and immune dysregulation syndrome (PLAID) (OMIM 614878) and autoinflammation, antibody deficiency, and immune dysregulation syndrome (APLAID) (OMIM 614878) (2-4) and familial cold autoinflammatory syndrome (FCAS3) (OMIM 614468) (5). All of them are autosomal dominant inherited diseases. Common variable immunodeficiency (CVID) is the most prevalent symptomatic heterogeneous group of primary immunodeficiency (PID) with low serum immunoglobulins and recurrent sinopulmonary infections mostly observed in the first decade of life (6). Recently, PLCG2 gene was found to be mutated in some of the CVID patients (1,6)

APLAID was characterized by recurrent blistering skin lesions with a dense inflammatory infiltrate and variable involvement of other tissues, including joints, eyes, and gastrointestinal tract. The patients had a mild humoral immune deficiency associated with recurrent sinopulmonary infections, but no evidence of circulating autoantibodies. Zhou et al. (3) noted that APLAID was a distinct disorder from PLAID, which they had described earlier (2), although both disorders shared impaired humoral immune function.

FCAS3 is an autosomal dominant immune disorder and autoinflammatory disease characterized by the development of cutaneous urticaria, erythema and pruritus in response to cold exposure. It is also characterized by the dysfunction of the inflammasome (5). From this point of view, FCAS3 patients usually display fever and inflammation at different organs, including the skin, joints, central nervous system and gastrointestinal

tract (5). Affected individuals may have additional immunological defects, including antibody deficiency, decreased number of B cells, defective B cells, increased susceptibility to infection and increased risk of autoimmune disorders (2).

In addition to infectious complications in CVID patients, at least one third of the patients experience autoimmune, autoinflammatory, granulomatous and/or malignant complications (7). The very heterogenous presentation of CVID strongly suggests a collection of different disease entities with somewhat different pathogenesis and most likely diverse genetic etiologies (7). Massive gene sequencing technologies have favored the description of mutations in several genes, but only in 10 % of CVID patients (8). These monogenetic defects are: ICOS, TNFRSF13B (TACI), TNFRS13C (BAFFR), TNRFSF12 (TWEAK), CD19, CD81, CR2 (CD21), MS4A1 (CD20), (CD27), LRBA, CTLA4, PRKCD, NFKB1, NFKB2, PIK3CD, PIK3R, VAV1, RAC1, BLK, IKZF1 (IKAROS) , IRF2BP2 and finally PLCG2 (8).

By means of our clinical experiences and recently obtained genetic results in six patients presented below, we suggest that PLAID, APLAID and FCAS3 are same diseases. In addition, a very small proportion of CVID patients are also PLAID/APLAID/FCAS3 patients and all these cases have a disease-causing mutation in PLCG2 genes, so maybe it may be better to define all of them as "PLCG2 deficiency" patients.

PATIENTS:

Patient 1

A 12-year-old girl presented with a history of recurrent respiratory tract infections for a few years and with decreased immunoglobulin levels. Her parents were consanguineous and family history was not remarkable for PIDs. In her physical examination, purulent postnasal discharge, splenomegaly and fine crackels in lower lobes of both lungs (bilateral bronchopneumonia) were observed.

Routine blood tests showed low serum immunoglobulins and lymphocyte count for her age: IgG:616 mg/dl, (normal: 1075 ± 228 mg/dl); IgA:17 mg/dl, (normal: 125 +- 43 mg/dl); IgM:33 mg/dl, (normal: 110 +- 38 mg/dl) and absolute lymphocyte count:1320/μl, (normal >1500/μl). Lymphocyte subsets by flow cytometry were in the normal range, anti-tetanus IgG (0.31 UI/ml) and anti-pneumococcal IgG (5 mg/l) were in protective levels. There was a decreased percentage of CD19+CD27+ B cells (0.5%, normal: 1.5–6.2%). Screening tests for autoimmunity were all negative. *Streptococcus pneumoniae* and *Moraxella catarrhalis* were detected in sputum culture.

There were chronic changes secondary to recurrent pulmonary infections on chest X-ray. There was reticular infiltration in the middle and left lung lower lobes, and no bronchiectasis was observed in thorax CT. Abdominal ultrasonography revealed splenomegaly.

The case with lymphopenia, hypogammaglobulinemia, decreased memory B cell, splenomegaly and recurrent upper/lower respiratory tract infections diagnosed as CVID and intravenous immunoglobulin therapy (IVIG) once a month, prophylactic itraconazole and TMP-SMX treatments were started (Table-1). After five years of follow-up, it was observed that the frequency of infections decreased significantly and life quality of the patient was better than before.

Then, we decided to perform "targeted next generation sequencing (TNGS)" in order to understand her molecular pathology. TNGS workflow based on an Ion AmpliSeq Primary Immune Deficiency Research Panel was designed for sequencing 264 PID genes on Ion S5 Sequencer and showed a heterozygous c.2152A>C (p.Ser718Arg) mutation in PLCG2 gene (Table-2). Although, this mutation was reported as VUS (variant of unknown significance) in the INFEVERS and CLINVAR databases and had low score in PolyPhen-2, by means of remarkable clinical and laboratory findings and good response to IVIG treatment, we strongly suggest that it is disease causing. Nowadays, the patient is in good general condition, no major infectious episodes, autoimmunity manifestations and lymphoproliferation occurred. Now, her exact diagnosis is PLCG2 deficiency.

Patient 2-3 (siblings)

A 3-year-old boy admitted with a history of severe eczematous rash, recurrent bronchiolitis, acute otitis media and bronchopneumonia. His parents were non-consanguineous and family history was negative for PIDs. His physical examination at admission was normal except for a mild eczematous rash on both hands and arms.

His initial immunologic evaluation revealed low IgG (410 mg/dl, normal: 848 ± 208 mg/dl) and IgM levels (39 mg/dl, 98 +- 33 mg/dl), normal IgA (78 mg/dl, normal: 58 +- 26 mg/dl). In flow cytometric lymphocyte subsets analysis, he had normal numbers of CD3+ T cells (86%) and low CD19+ B cells (1,7%). Specific IgG antibodies against tetanus and hepatitis B vaccines were both undetectable. His other laboratory tests including leukocyte, lymphocyte, haemoglobin counts, liver and kidney function tests, serologic investigations for common viruses, autoantibodies such as antinuclear antibody, direct Coombs tests, and abdominal ultrasonography were all normal. In chest CT, he had chronic bronchitis and bronchiolitis.

When he was five years old, genetic analyses for RAG1 and BTK were performed and no disease causing mutations were detected in these genes which were known to be associated with severe combined immune deficiency and X-linked agammaglobulinemia. We began to give him intravenous immunoglobulin (IVIG) replacement therapy (0.5 gm/kg) with four-week intervals. During follow-up of seven years under IVIG therapy, he was extremely well and had never severe infections.

When this patient was 6 years old, his brother was born. On the 28th day of his birth, he was admitted to our hospital because of maculopapular rash spreading all over his body especially after bathing. His physical examination was normal except for maculopapular rash. His initial immunologic evaluation revealed normal numbers of CD3+ T cells (88%) and low CD19+ B cells (1,6%). During follow-up, in his laboratary investigations, low IgG (228 mg/dl, normal: 507 +- 193 mg/dl), low IgA (10 mg/dl, normal: 28 +- 16 mg/dl), and slightly low IgM levels (37 mg/dl, 67 +- 30 mg/dl) were found. He had hypogammaglobulinemia and B cell deficiency, therefore we began to give him IVIG replacement therapy (0.5 gm/kg) with four-week intervals. After beginning of IVIG therapy, his quality of life increased and skin and respiratory system manifestations recovered. He has been followed-up regularly for four years in our department.

The elder brother was diagnosed as CVID at the beginning. In addition to recurrent infections, he had skin manifestations such as urticaria, erythema and recurrent eczematous rash that we rarely observe in classic CVID patients. Immunoglobulin levels of his sibling was very low when he was one year old and did not increase to normal levels as he gets older. Then, we thought that these siblings had a PID associated with dermatologic findings. In TNGS genetic analysis, a heterozygous c.502A>G (p.Thr168Ala) mutation was found in PLCG2 gene in both them. This mutation was reported as VUS in CLINVAR databases, but was reported benign in the INFEVERS database and also Polyphen-2 score was 0.00. As a result, their early diagnosis was CVID+APLAID and now they are called PID due to PLCG2 deficiency (Table-2). We strongly suggest that this mutation is disease causing, not VUS or benign, because of our long-term observations about their diseases management.

Patient 4

A 5-year-old girl had visited a pediatric clinic because of recurrent abdominal pain, fever, joint pain and swelling of the face. In her investigations, p.Arg202Gln heterozygous mutation had detected in MEFV gene and steroid treatment had been given during the attacks. When she admitted to our center, she had prolonged fever, severe abdominal pain and arthralgia and artritis at different joints (Table-1). Her parents were non-consanguineous and family history was negative. He was not thought as famial mediterrenean fever (FMF), because the alteration in her MEFV gene is a polymorphism and heterozygous and never expected to cause such severe clinical symptoms.

Between the attacks, her laboratory evaluation including leukocyte, lymphocyte, haemoglobin counts, liver and kidney function tests, autoantibodies such as antinuclear antibody, complement levels and rheumatic factor, acute phase reactants such as CRP, ESR were all normal. There was no proteinuria in urine examination. Her eye examination and abdominal ultrasonography were normal. During attack periods, besides clinical findings, we always observed leukocytosis, high erythroid sedimentation rate (ESR), C-Reactive

protein (CRP) and serum amiloid A (SAA) levels lasting at least one week.

The next generation sequence analysis revealed compound heterozygous p.Try482His and p.Asn571Ser mutations in PLCG2 gene (Table-2). Although these mutations were reported as benign in the INFEVERS and CLINVAR databases, PolyPhen-2 score was 0.974 (probably damaging). Then, the patient was thought to have one of the autoinflammatory periodic fever syndromes and diagnosed as familial cold autoinflammatory syndrome-3 (FCAS3).

Canakinumab was administered monthly for 6 months (initial treatment), bimonthly for 6 months (maintenance treatment), then treatment was discontinued. The patient developed a new attack one-year after discontinuation of treatment period, canakinumab began to be readministered with 3-month intervals (continuation treatment). Canakinumab was highly effective and the patient was completely recovered and during two years of follow-up she had never above clinical symptoms suggesting us that her FCAS3 diagnosis is exactly correct.

Patient 5

This 11-year-old male patient is the second child of nonconsanguineous, healthy parents. He had admitted to the pediatric infection department with respiratory infections and recurrent skin infections namely fronculus when he was 9 years old. The patient, who was given intravenous antibiotic treatment previously, was referred to us in terms of PID. His physical examination was normal. His initial immunologic evaluation revealed normal IgG (940 mg/dl, normal: 1088 +- 238 mg/dl), IgA (134 mg/dl, normal: 124 +- 45 mg/dl), and low IgM levels (28 mg/dl, 104 +- 49 mg/dl). He had normal numbers of CD3+ T cells (82%, 1508/mm3) and CD19+ B cells (9.6%, 176/mm3) and normal numbers and percentages of other lymphocyte phenotypes (CD3+CD4+ T helper cells 35% and 644/mm3, CD3+CD8+ T cytotoxic cells 35% and 644/mm3, CD3-CD16+CD56+ natural killer cells 7.6% and 140/mm3). His other laboratory tests including leukocyte, lymphocyte, haemoglobin counts, liver and kidney function tests, serologic investigations for common viruses were all normal.

Specific IgG antibodies against *Haemophilus influenza type B* and*pneumococcus* vaccines were undetectable. The patient was diagnosed as "specific antibody deficiency". Specific antibody deficiency (SAD) is a PID characterized by recurrent respiratory system infections, normal immunoglobulin (IG) and IgG subclass levels, and poor response to polysaccharide vaccinations in children older than 2 years of age. We decided to perform "targeted next generation sequencing (TNGS)" in order to determine the possible genetic causes of this immune deficiency and a hemizygous c.415C>T (p.Pro139Ser) mutation was found in PLCG2 gene. This mutation was reported as VUS in the INFEVERS and CLINVAR databases and very high PolyPhen-2 score (0.977) (Table-2). He has been succesfully followed-up by using prophylactic antibiotics for two years in our clinic and his recent and exact diagnosis is "PLCG2-associated antibody deficiency and immune dysregulation syndrome (PLAID)".

Patient 6

The 8-year-old girl referred to our center due to the skin rash existing for one month on the whole body. The rash had regressed with oral antihistaminic and corticosteroid treatments, and recurred after 2 weeks. Her parents were consanguineous and family history was negative for PIDs and allergic diseases. Her physical examination was normal except for multiple ovaloid scars on the extremities and cutaneous urticaria, erythema and signs of pruritus on the other parts of the body. Her initial immunologic evaluation revealed high IgG (1240 mg/dl, normal: 1061 +- 203 mg/dl), IgA (169 mg/dl, normal: 116 +- 42 mg/dl) and normal IgM levels (86 mg/dl, 106 +- 42 mg/dl). Although serum IgE concentration was extremely high,1106 IU/mL (normal level is 0 - 15 IU/mL), mix multiple allergen-specific IgE was 0.06 ku/L (normal level: $<$ 0.35 ku/L). There was no peripheral eosinophilia. Lymphocyte subsets by flow cytometry were in the normal range, specific IgG antibodie against hepatitis B vaccine was detectable. Her other laboratory tests including C3, C4, C1 inhibitor levels, rheumatic factor, serologic investigations for common viruses, autoantibodies such as antinuclear antibody and anti-neutrophil cytoplasmic antibodies were all normal. Although urinary analysis showed abundant leukocytes, urine culture was sterile. She had no proteinuria and no parasites

were observed in feces. There were findings compatible with vasculitis in skin biopsy (Table-1).

Primary Immune Deficiency Research Panel sequencing 264 PID genes was performed to find if there is a gene causing hyper IgE syndrome. However, only a heterozygous p.Arg268Ala mutation was found in PLCG2 gene (Table-2). Although this mutation was reported as benign in the INFEVERS and CLINVAR databases, she was diagnosed as APLAID syndrome, because of her genetic result, cold exposure skin findings, vasculitis, high IgE and partial response to montelukast and ketotifen therapy. Total IgE slightly decreased (414 IU/mL) in seven years of follow-up.

DISCUSSION

In 2009; Gandhi et al (9) reported thirty-five subjects who were described as familial atypical cold urticaria (FACU) displaying an autosomal dominant pattern of inheritance. All affected subjects had lifelong symptoms that began in early childhood with pruritus, erythema, and urticaria. A history of atopy was reported in 14% of patients. Most of the patients tested showed immunologic defects, including antibody deficiency (75%), recurrent infections (56%), and autoantibodies or autoimmune disease (56%) (9). Laboratory studies showed decreased serum IgA and IgM, decreased circulating B cells, decreased memory B cells, and decreased natural killer cells (9). Indeed, some of the initially published patients with PLAID and the ones reported by Gandhi et (9) above fulfilled the diagnostic criteria of CVID. This phenotypical overlap might be explained by aberrant PLCG2 signaling downstream of the B cell receptor and Fcγ receptors on B cells (6). In addition, it has been reported that defects in PLCG2 gene cause functional disorders in Bruton tyrosine kinase (BTK) enzyme leading to B cell developmental delay and decreased antibody production (10). Szymanski and Ombrello (11) have reported that signaling abnormalities in macrophages and neutrophils caused by PLCG2 gene defects may also contribute to the pathogenesis of granulomatosis in PLAID.

Immune deficiency with increased susceptibility to infection is commonly observed in PLAID, where B lymphocyte abnormalities (patient 2 and 3) and low serum IgG, IgM and/or IgA levels (patients 1-2-3) are the most common features as they were diagnostic criteria of CVID (11). Our first, second and third patients had the diagnosis of CVID and successfully treated with IVIG replacement. Qualitatively, many PLAID patients demonstrate reduced antibody responses to specific stimuli, such as pneumococcal antigens (11). Our 5th patient had specific antibody deficiency (SAD) and PLAID diagnosis. Hajjar et al (12) reported that prophylactic antibiotics and immunoglobulin replacement therapy are equally effective as first line in preventing infections in SAD patients. Our patient responded very well to prophylactic antibiotics and he was prevented from recurrent bacterial infections.

PLAID patients have normal numbers of T cells, but almost all have low numbers of circulating class-switched CD27+ memory B lymphocytes (11). Our first patient had also decreased CD27+ B cells. Additionally, in Szymanski and Ombrello's study, 3 of 27 PLAID patients had been diagnosed with CVID and had been treated with IVIG for severe, recurrent pneumonia with bronchiectasis (11). Similarly, our three patients were regularly treated with IVIG. This is important because of the striking phenotypic overlap between PLAID and CVID.

Symptomatic allergic disease is present in most people with PLAID/APLAID, including asthma, eczema, allergic rhinitis and food allergies (11). Most of the patients had increased IgE (9). Our sixth patient had high IgE, but specific allergy tests for environmental and food allergens were negative and the patient had urticaria and erythema, not the respiratory allergic symptoms. Antihistamines did not provide fully relief. Autoimmunity is also commonly found, with two-thirds of patients having positive antinuclear antibody testing (11). However, none of our patients had positive antinuclear antibody.

Discovering the genetic causes of monogenic autoinflammatory diseases permitted their recognition as disorders and many of them are mediated by the release of proinflammatory cytokines such as interleukin-1β (IL-1β) (13). FCAS3 is one of them and its disease causing monogene is PLCG2. PLCG2 is expressed in lymphocytes as well as innate immune cells, and is known to trigger a number of signaling pathways, including protein kinase C (13). Fever and inflammatory symptoms are predominant in FCAS3 patients and one would expect to have a dramatic response to IL-1 inhibitors such as anakinra and canakinumab (13).

Our fourth patient is exactly diagnosed as FCAS3 by means of her relevant clinical findings and p. P139S heterozygous mutation with extremely high PolyPhen-2 score (0.977). Her very good response to canakinumab therapy also supports our suggestions.

Conclusions : Several striking phenotypes can emerge from PLCG2 disorders, and the pathophysiology leads to a complex mix of loss and gain of function in cellular signalling (14). These phenotypes may highly overlap or may be somewhat different. For example, unlike in PLAID, the APLAID patients do not have substantial autoantibody formation (14). Why the point mutations in the same region lead to only minimal or high overlap has yet to be understood. However, we believe that PLAID/APLAID/FCAS3 patients must have the same and unique name as "PLCG2 deficiency" in future studies. The frequency of PLCG2 deficiency in CVID patients needs to be determined and these cases also be mentioned as "PLCG2 deficiency", not CVID.

The last conclusion is although some of these mutations are reported as bening or VUS in INFEVERS and CLINVAR data and have low Polphene-2 scores, by means of very important and severe clinical findings and succesfull response to therapies, we suggest all of them are pathogenic and disease causing. Unfortunately, we are not able to do functional studies about these genetic alterations. We are advising to revise their damaging effects in databases.

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Author contributions: NK designed the study and wrote the paper. EY wrote the paper and provided clinical information and the samples. AA, AD, OC and AB performed the genetic analysis. NEK, GA and RBGB performed clinical follow-up of patients.

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Hy7bPatient No/ID	**Gender**	**Consanguinity**	**Age (year)**	**Age at admission (year)**	**Remarkable clinical findings**	**Laboratory findings**	**Therapy**
1/S.E.E	Female	2nd degree	17	12	-Recurrent respiratory tract infections -Splenomegaly	-Hypogammaglobulinemia -Lymphopenia -decreased memory B cell	-IVIG replacement -Itraconazole / TMP-SMX prophylaxis
2/B.K.A	Male	(-)	10	3	-Recurrent eczematous rash and sinopulmonary infections -Chronic bronchitis and bronchiolitis	-Hypogammaglobulinemia -B cell lymphocytopenia -Urticaria and erythema	-IVIG replacement
3/E.A	Male	(-)	4	1st month	-Maculopapular rash in the neonatal period	-Hypogammaglobulinemia -B cell lymphocytopenia -Urticaria and erythema	-IVIG replacement

Hy7bPatient No/ID	Gender	Consanguinity	Age (year)	Age at admission (year)	Remarkable clinical findings	Laboratory findings	Therapy
4/E.B.D	Female	(-)	7	5	- Recurrent severe abdominal pain, prolonged high fever, joint pain and swelling of the face	(-)	- Canakinumab
5/E.T	Male	(-)	11	9	- Recurrent fronculus and respiratory infections	-Specific antibody deficiency	- Prophylactic antibiotics
6/S.T	Female	3rd degree	15	8	- Cutaneous urticaria, erythema, pruritus - Vasculitis (skin biopsy)	- High IgE	- Montelukast and ketotifen

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Table-1: Demographic, remarkable clinical and laboratory finding of the study group

Table-2: PLCG2 mutations and initial diagnosis of the patients

Patient no/ID	PLCG2 mutation	PolyPhen-2	INFEVERS	CLINVAR	Early Diagnosis	Diagnosis: Primary immunodeficiency due to
1/Ş.E.E	p.S718R heterozygous mutation	BENIGN Score: 0.171	VUS	VUS	**CVID**	**PLCG2 defect**
2/B.K.A	p.T168A heterozygous mutation	BENIGN Score: 0.00	BENIGN	VUS	**CVID + APLAID**	**PLCG2 defect**

Patient no/ID	**PLCG2 mutation**	**PolyPhen-2**	**INFEVERS**	**CLINVAR**	**Early Diagnosis**	**Diagnosis: Primary immunodeficiency due to**
3/E.A * Patients 2 and 3 are siblings	p.T168A heterozygous mutation	BENIGN Score: 0.00	BENIGN	VUS	**CVID + APLAID**	**PLCG2 defect**
4/E.B.D	p.Y482H/p.N571S compound heterozygous mutation	PROBABLY DAMAGING score: 0.974 /BENIGN score: 0.001	BENIGN	BENIGN	**FCAS3**	**PLCG2 defect**
5/E.T	p. P139S Heterozygous mutation	PROBABLY DAMAGING score: 0.977	VUS	VUS	**PLAID**	**PLCG2 defect**
6/S.T	p. R268A heterozygous mutation	BENIGN score: 0.055	BENIGN	BENIGN	**APLAID**	**PLCG2 defect**