

# Leukocyte Adhesion Deficiency-I with a Novel Intronic Mutation Presenting with Pyoderma Gangrenosum- Like Lesions

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**Abstract** Pyoderma gangrenosum (PG) is an uncommon noninfectious neutrophilic dermatosis characterized by recurrent, sterile, necrotic skin ulcers. It is commonly associated with underlying systemic disease like inflammatory bowel disease, rheumatoid arthritis and hematological malignancies. Pathogenesis of PG remains unclear though aberrant immune responses have been implicated. The diagnosis of PG is of exclusion and management is empirical with local or systemic immunosuppressive therapy. LAD-I is a rare form of autosomal recessive disorders caused by mutations of the gene *ITGB2*, clinically characterized by recurrent severe bacterial infection, impaired pus formation, poor wound healing and persistent neutrophilia. Though skin ulcerations are common, predominant clinical presentation as PG is unusual in LAD-I. Here we present four Indian patients with LAD-I from three unrelated families initially diagnosed as PG due to chronic recurrent skin ulcerations requiring steroids and antibiotics for healing, associated with atrophic scar formation. All these four patients had persistent neutrophilia without history of delayed cord separation and showed moderate expression of CD18 (19 to 68 %) on neutrophils. Sequencing of the entire coding region and intronic splice sites of the *ITGB2* gene from the genomic DNA of these patients revealed a novel common mutation IVS10+4A>G. LAD-I should be kept in mind while evaluating patients with PG especially those with persistent

neutrophilia in the absence of other rheumatological disorders. Diagnosis of LAD-I in these cases is extremely important for management as treating these patients without adequate antibiotic cover may prove fatal and these patients often require hematopoietic stem cell transplantation for permanent cure.

**Keywords** LAD-I · pyoderma gangrenosum · *ITGB2* gene mutation · primary immunodeficiency disorder

## Introduction

Pyoderma gangrenosum (PG) is an uncommon ulcerative skin disease characterized by primarily sterile, painful, enlarging necrotic ulcers with bluish undermined borders surrounded by advancing zones of erythema [1–3]. The pathogenesis for this chronic inflammation is unclear, but an aberrant immune response to yet unidentified antigen is the most widely accepted theory. Around 50 % of the cases of PG are associated with underlying systemic disease, most commonly inflammatory bowel disease, rheumatoid arthritis and hematological malignancies and the remaining are idiopathic [4]. The peak of incidence occurs between the ages of 20–50 years with women being affected more often than men [5].

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LAD-I is a rare form of autosomal recessive disorders caused by mutations of the gene *ITGB2*, encoding for the common  $\beta$ -chain of the  $\beta 2$  integrin family, designated as CD18 [6, 7]. Defects in *ITGB2* gene affect the leukocyte trafficking mediated by adhesive interactions between myeloid leukocytes and inflamed endothelial cells, critical for defense against bacteria and wound healing. Patients with severe phenotype have <2 % of CD18 expression (LAD-I<sup>0</sup>) whereas >2–20 % (LAD-I<sup>−</sup>) expression have moderate phenotype. Rarely patients may have >20 % or near normal expression of CD18 (LAD-I<sup>+</sup>), however, it is functionally abnormal and present with mild clinical phenotype [8]. LAD-I is characterized by leukocytosis, recurrent infections, omphalitis with delayed cord separation, impaired pus formation and slow wound healing [9]. Necrotic skin ulcerations especially around perianal area due to Gram negative organisms are common in severe LAD-I however they are almost always associated with systemic manifestations like sepsis. Here we present four cases of LAD-I with mild CD18 deficiency presenting predominantly with PG-like lesions.

## Patient Presentation

Four patients (3-males, 1-female) presented with recurrent skin ulcerations, since 2 years of age. The diagnosis of LAD-I was significantly delayed (6–16 years of age) in all. The ulcers usually began with small pustules or after a trivial trauma, and rapidly progressed to necrotic lesions, affecting mainly lower limbs however were also seen on other parts of the body. Wound healing was delayed and always required long term antibiotic and sometimes systemic immunosuppressive therapy. The healing was with thin atrophic scar formation (Fig. 1). Though a significant morbidity was observed other clinical manifestations like delayed cord separation, omphalitis, periodontitis, recurrent systemic infections were absent. The growth and development of these patients was within normal limits. Though ulcer swabs of two of these patients grew *pseudomonas aeruginosa* on one occasion each, most of the other swabs showed no growth of organism. P1 and P2 patients required multiple courses of oral and intravenous antibiotic therapy and topical antibiotic applications. P3 and P4 were treated with antibiotics however the ulcers also required courses of system steroids along with systemic antibiotics for wound healing. Skin biopsy of patients P2 and P1 showed infiltration with neutrophils as well as lymphocytes. Skin biopsy of P4 showed focally ulcerated epidermis with sub epidermal cleft formation, mononuclear infiltrate with scattered giant cells and relative paucity of neutrophils (Fig. 1). The hematological parameters and CD18/11 expression are described in Table 1. They showed persistent neutrophilia

with moderate CD18/11 expression. One patient (P3) had a history of consanguinity. P3 also had a history of similar illness in paternal cousin sister who expired at the age of 15 years.

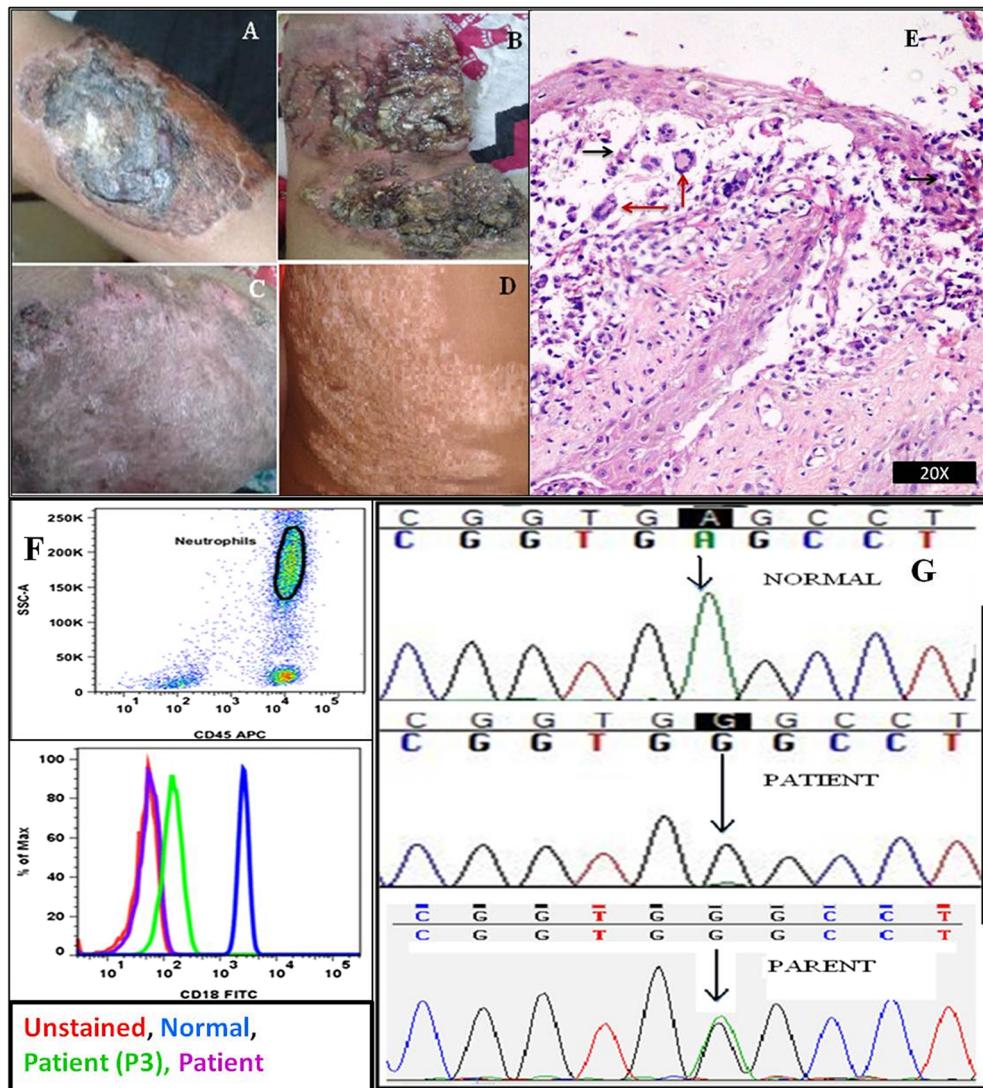
The entire coding and intronic splice sites region of the *ITGB2* gene were sequenced from genomic DNA. All patients showed common IVS10+4A>G mutation (3-homozygous, 1-heterozygous) in Intron 10 splicesite region. Parents also showed this mutation in the heterozygous state. It was absent in 30 normal samples screened. Insilico analysis was done using Alamut software (Interactive Biosoftware, Rouen, France), GeneSplicer [10], Human Splicing Finder [11], MaxEntScan [12], NNSPLICE [13] and SpliceSite Finder, [14].

## Discussion

Busting et al. first coined the term ‘pyoderma gangrenosum’ to describe patients with rapidly progressive, painful, suppurative cutaneous ulcers with edematous, boggy, blue, undermined and necrotic borders [15]. PG is a condition associated with recurrent skin ulcerations with noninfectious neutrophilic infiltrates. It is uncommon in children with only 4 % being infants and adolescents [1]. Some autoinflammatory disorders like PAPA syndrome are seen in pediatric age group presenting with PG but are also associated with pyogenic arthritis, and acne [16]. Large number of ulcerative skin conditions including infections and drugs mimic PG and their exclusion is essential for diagnosis of PG. Misdiagnosis is very common, seen in up to 30 % of the cases and it exposes the patient to the substantial risks associated with its treatment including delay in diagnosis [17].

LAD syndromes result from failure of leukocyte to defend the host because of missing or dysfunctional surface adhesion molecules [18].  $\beta 2$  integrins are expressed selectively by leukocytes and participate in many functions related to immune defense against pathogens, including leukocyte adhesion and transendothelial migration, phagocytosis and cell-mediated cytotoxic activity. Life threatening infections such as septicemia, bronchopneumonia and meningitis result in significant mortality seen in these cases. Though skin ulcerations are common, predominant presentation with PG in absence of other features like periodontitis and systemic infections is rare and has been described in few patients with partial CD18 deficiency [19–22]. Though, clinically the ulcers in LAD-I simulate PG but histologically they can be differentiated from true PG ulcers, as these show ‘relative paucity of neutrophils in dermis’ as described in multiple reports [20, 21]. This in contrast to true PG ulcer where histopathology shows predominant and abundant neutrophilic infiltrate in the dermis as well as in subcutis, even the ‘neutrophilic abscesses’ are formed in the dermis. In this series P4 showed paucity of

**Fig. 1** **a** and **b**: Photographs of partially treated ulcers of patients P3 and P4 respectively. **c** and **d**: Photographs of wound healing with thin atrophic scar formation in patient P3 and P4. **e**: H & E 20X micrograph of PG like ulcer. Black arrows show focally ulcerated epidermis with sub epidermal cleft formation. Red arrows show scattered giant cells. Mononuclear infiltrate are shown in basophilic dots and relative paucity of neutrophils can be seen. **f**: Expression of CD18 on neutrophils: Dot plot showing neutrophils gating on side scatter and CD45. Histogram shows expression of CD18 in normal (blue), moderate CD18 expression in patient-P3 (green) whereas complete absence of CD18 in a severe LAD patient is shown in violet. **g**: Novel splice-site mutations in *ITGB2* gene IVS10+4A>G seen in all the four patients in homozygous form and heterozygous form in asymptomatic parents



neutrophils in the skin biopsy specimen where as P1 and P2 showed presence of both neutrophils as well as lymphocytes on histopathology. The presence of neutrophils within the lesions may indicate partial neutrophil recruitment potential in these patients. Swab cultures from these ulcers often do not grow any organism as these patients are on chronic antibiotic therapy. A partial or temporary improvement of ulcers after immunosuppressive therapy may lead to misdiagnosis of PG in these cases [19]. Moreover treatment with high dose of steroids without antimicrobial therapy may prove life-

threatening and hence diagnosis of underlying condition becomes extremely important [19].

A recent study however has shown that periodontal disease in LAD-I patients is driven by excessive IL-17 dependent inflammatory responses [23]. Two of these patients P3 and P4 also required prolonged therapy with systemic steroids along with antibiotic therapy to control the inflammation. Unfortunately the cytokine profile was not done in any of these patients. This may suggest possibility of deregulated immune response in pathogenesis of these PG-like lesions

**Table 1** Clinical features in LAD-I<sup>+</sup> cases

Patients	Gender	Age at diagnosis	Consanguineous marriage	Absolute neutrophils count	CD18/CD11 %	Phenotype
P1	Male	4 years	No	$43.5 \times 10^3$	68 %	LAD-I <sup>+</sup>
P2	Female	11 years	No	$11.9 \times 10^3$	45 %	
P3	Male	2 years	Yes	$19.3 \times 10^3$	50 %	
P4	Male	16 years	No	$23.4 \times 10^3$	19 %	

seen in LAD-I. Although milder forms of LAD-I patients survive till adulthood with good supportive care, in some of them quality of life is poor and they require hematopoietic stem cell transplantation for permanent cure. Patient P4 has undergone stem cell transplant and is currently asymptomatic.

Interestingly all four patients showed a same mutation IVS10+4A>G with partial expression of CD18. 2 of the 4 patients (P1 and P2) were siblings but other two were unrelated and all 3 families were from different ethnic background. Functional insilico analysis of this mutation using different software showed significant reduction in the strength of splicing at the donor site and in parallel creation of the new splice site for splicing enhancer SF2/ASF (IgM-BRCA1) and SF2/ASF in the IVS10+4 region. Thus this splice site defect though located in the less conserved region is likely to be pathogenic and results in the partial expression of CD18. Aberrant integrin [CR4;  $\alpha\beta 2$ ; CD11c/CD18] oscillation on neutrophils and aberrant neutrophil trafficking has been demonstrated in patients with PG [24, 25] and this partial but dysfunctional CD18 expression may be responsible for the predominant PG like picture seen in these patients.

LAD-I<sup>+</sup> can present as PG-like lesions and must be suspected in patients with PG especially when presenting below the age of five years and associated with atrophic scar formation and persistent neutrophilia. The accurate diagnosis in these patients is important as they cause significant morbidity and the severe ones can be considered for HSCT for permanent cure.

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