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Beyond Dermatological Findings: Multisystem Involvement in Prolidase Deficiency

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What is already known on this topic?

- *Prolidase deficiency is a metabolic and immunological disorder that is inherited in an autosomal recessive manner.*
- *In prolidase deficiency, a broad spectrum of differences is observed in patients, ranging from asymptomatic to multisystem involvement, with dermatological symptoms often being predominant.*

What this study adds on this topic?

- *Recurrent opportunistic infections, gastrointestinal involvement, and malignancies can be observed, highlighting the atypical nature of the clinical presentation.*
- *Flow cytometry findings indicative of combined immunodeficiency can be detected in some patients, which can be helpful for the early suspicion of prolidase deficiency.*

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ABSTRACT

Objective: Prolidase deficiency is a metabolic and immunological disorder that is inherited in an autosomal recessive manner. In prolidase deficiency, a broad spectrum of differences is observed in patients, ranging from asymptomatic to multisystem involvement. There is scarce information in the literature on the atypical features and immunophenotypes of this disease. Aim of this study is to present 4 new cases to provide information on the rare features of the disease and to raise awareness.

Materials and Methods: This study included 4 female patients with prolidase deficiency. Their demographic, clinical, and immunologic characteristics were obtained from their medical records.

Results: There were 4 female patients (P1-P4), with a mean age of 18.5 years (min-max: 10-29) and a mean age of symptom onset of 6.9 years (min-max: 0.04-27). The main presenting complaints of the patients were skin lesions (100%), dysmorphic features (100%), neurodevelopmental delay (100%), frequent infections (100%), and prolonged diarrhea (50%). P2 had diffuse large B-cell lymphoma, resulting in early death. Interestingly, P1 and P2 experienced opportunistic infections such as cytomegalovirus, Epstein-Barr virus, and *Pneumocystis jirovecii*. Three patients (75%) had lymphopenia. Two patients had elevated IgE levels. Lymphocyte subgroup analysis showed an inverted CD4/CD8 ratio in all patients. In patients P1 and P2, the percentages of naive T cells and recent thymic emigrants were reduced, suggesting combined immune deficiency at the time of diagnosis. $CD19^+$ B cells were also low in P1 and P2. Metabolic evaluations revealed low prolidase enzyme activity in P1 and P2.

Conclusion: Beyond the well-known classical dermatological findings, the presence of recurrent opportunistic infections, gastrointestinal involvement, malignancy, and flow cytometry findings suggestive of combined immunodeficiency indicate that the diagnosis of prolidase deficiency may be underestimated. Knowing the atypical and rare presentations will facilitate diagnosis and treatment of affected patients.

Keywords: Immunodysregulation, lymphoma, prolidase deficiency, PEPD gene

INTRODUCTION

Inborn errors of immunity (IEI) represent a group of rare disorders characterized by a wide range of symptoms, including unusually severe infections, increased cancer susceptibility,

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and exaggerated inflammatory responses that can impair organ function. The spectrum of these immune disorders is broad, with presentations ranging from mild infections to severe multisystem disease.[1]

Prolidase deficiency is an autosomal recessive metabolic disorder caused by biallelic mutations in the *PEPD* gene, which encodes the enzyme prolidase (also known as peptidase D). This deficiency leads to impaired degradation of collagen and other proline-containing proteins. First described by Goodman[2] in 1968, approximately 160 cases of prolidase deficiency have been reported in the literature.[3] In 2019, the International Union of Immunological Societies (IUIS) classified prolidase deficiency as a disease of immune dysregulation. It was placed in the subgroup of autoimmune diseases and included in the IEI.[4,5]

Prolidase is a dipeptidase responsible for hydrolyzing peptide bonds in dipeptides that contain proline or hydroxyproline at the C-terminal position. The enzyme functions as a dimer and requires manganese as a cofactor. Prolidase is involved in the rate-limiting step of collagen recycling and plays a critical role in protein metabolism, collagen turnover, and matrix remodeling. Consequently, it is essential for processes such as wound healing, inflammation, angiogenesis, and carcinogenesis.[6] Mutations in the *PEPD* gene lead to a deficiency in prolidase activity, which impairs wound healing and contributes to characteristic skin manifestations.[7]

Prolidase deficiency exhibits a broad clinical spectrum, with presentations ranging from asymptomatic cases to multisystem involvement. Common clinical features include chronic skin ulcers (often on the lower extremities), telangiectasias, dysmorphic facial features such as a prominent forehead, hypertelorism, ptosis, ocular proptosis, exophthalmos, slanted palpebral fissures, a small nose with a low nasal root, a beaked nose, and a slender upper lip. Additionally, patients often experience delayed neurodevelopmental milestones, splenomegaly, recurrent upper respiratory tract and skin infections, and hematological abnormalities such as anemia and thrombocytopenia. Chronic lung diseases and systemic lupus erythematosus have also been increasingly associated with prolidase deficiency.[3,8,9]

Mental retardation is frequently observed in individuals with prolidase deficiency, which may be attributed to elevated concentrations of proline, a compound known to disrupt glutamatergic neuronal function in the central nervous system.[10]

In this report, the demographic, clinical, and immunological characteristics of 4 patients with prolidase deficiency were described to further expand the clinical and immunological spectrum of the disease and to provide comprehensive insights for early diagnosis and treatment.

MATERIALS AND METHODS

The study included 4 patients with prolidase deficiency. Their demographic, clinical, and immunologic characteristics were obtained from their medical records. The medical records of the patients were examined in detail. A questionnaire containing demographic and clinical data (age of onset of symptoms, age at diagnosis, duration of follow-up, family history, previous infections, systemic involvement, genetic features, diagnostic examination, treatments, and clinical course) was completed for each patient. Peripheral lymphocyte subgroup analyses were measured by flow cytometry, as described previously.[11-13] The results were evaluated by considering the reference values of Turkish children and adults.[14] Serum immunoglobulin levels were measured by nephelometry. The genetic diagnosis was made by next-generation sequencing with Sanger validation.

The Marmara University Faculty of Medicine Ethics Committee approved the study protocol under protocol number 2013-0200 and date June 28, 2016. Written informed consent was obtained from all patients' legal guardians before initiating any study procedure. Families of each patient provided written informed consent, and all studies were conducted in accordance with the principles of the Declaration of Helsinki.

The prolidase activity level was calculated using the average absorbance values obtained from serum, defined as the amount of proline produced per liter per minute and the enzyme quantity that modifies 1 micromole of substrate per minute.[15] The formula used for calculation is as follows: Prolidase activity level (units/liter) = (A−B) × (S) × F × 1000 / S where A represents the sample absorbance, B represents the blank absorbance, S is 1000, and F is the standard absorbance.

Statistical Analysis

Statistical analysis of the data was conducted using Jamovi version 2.3.26 (The Jamovi Project, Sydney,Australia). Figures were created using Adobe Illustrator 25.2.1 (Adobe Inc., San Jose, California) and GraphPad Prism version 9.0.0. (GraphPad Software Inc., San Diego, California).

RESULTS

Demographic and Clinical Findings

Four female patients (P1, P2, P3.1, and P3.2) from 3 different families were included in the study, with a mean age of 18.5 years (min-max: 10-29) and a mean age of symptom onset of 6.9 years (min-max: 0.04-27). The demographic and clinical characteristics of the patients are presented in Table 1 and Figure 1, with detailed clinical summaries available in the Supplementary file. Consanguinity was present in the families of 3 patients, while the parents of 1 patient (P2) were from the same village. All patients were of Turkish origin.

Except for the siblings P3.1 and P3.2, no family history of similar diseases was reported. The main complaints at the time of initial presentation were skin lesions (100%), frequent infections (100%), and prolonged diarrhea (50%; P1 and P3.1). By the end of the study, 2 patients (P3.1, P3.2) were still alive, with a mean follow-up duration of 11.2 years (min-max: 2.7-22.2). Patient P1, who experienced frequent hospitalizations due to sepsis, died at age 10 from multiorgan dysfunction secondary to sepsis. P2, diagnosed with diffuse large B-cell lymphoma (DLBCL, grade 4B) and treated with the R-CHOP chemotherapy protocol (Rituximab-cyclophosphamide-doxorubicin-vincristine-prednisone), passed away at age 29 due to sepsis, respiratory distress, and multiorgan dysfunction after the first cycle of treatment.

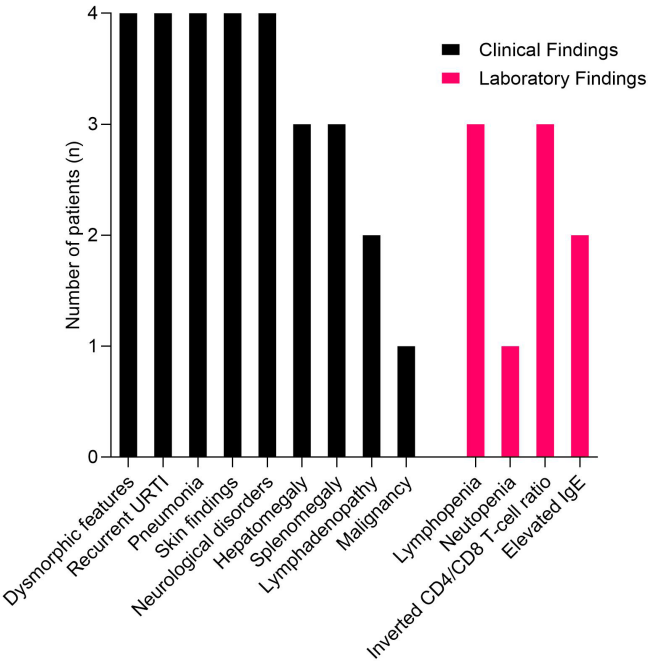

Figure 1. Common clinical findings and laboratory results in prolidase deficiency.

Dysmorphic Features

All patients (100%) exhibited dysmorphic features, as summarized in Table 1 and shown in Figure 2. Three patients had height and weight percentiles below the 3rd percentile, while P2 did not exhibit growth retardation.

Skin Findings

P1 presented with severe diaper dermatitis in infancy. By age one, desquamation on an erythematous base developed on the dorsal surfaces of both hands, with papular lesions on the elbows and xerotic erythematous lesions. P2, despite having no previous skin lesions, presented at age 27 with widespread tinea corporis affecting the face, trunk, and legs. This tinea corporis was extensive and resistant to antifungal treatment, regressing only after 2 months of local and systemic therapy. However, a lesion on the anterior leg persisted, becoming ulcerated (Figure 3). A skin biopsy was performed to differentiate between deep fungal infection, granulomatous disease, vasculitic lesion, and cutaneous lymphoma. The biopsy showed hyperkeratosis, acanthosis, follicular epithelium destruction, neutrophil and leukocyte infiltration, and fungal hyphae and spores. Trichophyton tonsurans/rubrum (dermatophyte) grew from a wound swab culture. Although antifungal treatment led to regression of the leg lesions, residual changes persisted, and papular lesions on the hands did not regress. Recurrent cutaneous mycotic infections were observed throughout the follow-up.

In P3.1 and P3.2, widespread erythematous, crusted, erosive, and occasionally ulcerative lesions were observed on both legs. These lesions first appeared in P3.1 at age 3. A skin biopsy from the anterior leg showed histological signs of subacute-chronic spongiotic dermatitis consistent with atopic dermatitis, though no bacterial growth was detected. Despite treatment with local steroids and antibiotics, the lesions persisted, and symptomatic treatment was provided. Both patients continue to exhibit erythematous ulcerative lesions on the anterior surfaces of their legs. The skin lesions are summarized in Table 1.

Infections

Prolidase deficiency commonly presents with recurrent sinopulmonary infections. All 4 patients had a history of recurrent bronchiolitis during infancy. Additionally, 3 patients (P1, P3.1, P3.2) experienced recurrent bronchopneumonia starting before 6 months of age, which required hospitalization.

Patient P1 was hospitalized at 3 months due to cytomegalovirus (CMV) pneumonia and received ganciclovir treatment. Patient P2, a 27-year-old female, presented with a prolonged fever and skin lesions. Initial empirical antibiotic therapy was ineffective. A chest computed tomography scan revealed bilateral ground-glass opacities (Figure 4), and bronchoalveolar lavage was positive for Pneumocystis jirovecii pneumonia (PCP) via PCR. Treatment with a 21-day course of trimethoprim-sulfamethoxazole and steroids resulted in clinical and radiological improvement. Given the patient's CD4 lymphopenia, prophylactic trimethoprim-sulfamethoxazole and immunoglobulin replacement therapy was initiated. Bone marrow aspiration revealed hypergranulation, indicative of chronic infection, with no evidence of malignancy. Suspected lymphoma prompted PET scans, which showed multiple FDG-avid lymphadenopathies. A lymph node biopsy showed no malignancy. Despite recurrent lymphadenopathy and mycotic abscesses, excisional biopsies from the inguinal and cervical regions ruled out lymphoma. However, 1 year later, a cervical biopsy confirmed a diagnosis of DLBCL. The patient began R-CHOP chemotherapy but developed a neutropenic fever during the first cycle, requiring intensive care admission. She also experienced epileptic seizures, presumed to be secondary to lymphoma involvement. Unfortunately, the

Table 1. The Demographic, Clinical and Syndromic Manifestations of PEPD-Deficient Patients

Evaluated parameters	**P1**	**P2**	**P3.1**	**P3.2**
Initial symptoms	Diarrhea, frequent infections	Skin lesions, fever	Skin lesions, frequent infections	Frequent infections
Variant	c.220del, p.Ala74ArgfsTer56	c.796A>G, p.Thr266Ala	c.1359_1361del p.Glu453del	c.1359_1361del p.Glu453del
Alive/dead (age at death)	Dead (10 yrs)	Dead (29 yrs)	Alive	Alive
Cause of death	Sepsis, multiorgan failure	Non-Hodgkin Lymphoma	–	–
Head & Neck				
Face				
Prominent forehead	+	+	–	–
Facial dysmorphism	+	+	+	+
Eyes				
Hypertelorism	+	–	+	+
Ptosis	+	–	–	–
Ocular proptosis	+	–	–	–
Exophthalmos	+	–	–	–
Upslanting palpebral fissures	+	–	–	–
Downslanting palpebral fissures	–	+	+	+
Nose				
Small nose	+	–	+	+
Low nasal root	+	+	+	+
Baked nose	–	+	+	+
Mouth				
Slender upper lip	+	–	+	+
Respiratory				
Lung				
Recurrent pulmoner infections	+	+	+	+
Chronic Lung Disease	+	+	+	+
Abdomen				
Hepatomegaly	+	+	+	–
Splenomegaly	+	+	+	–
Skin, Nails, & Hair				
Skin				
Crusting erythematous dermatitis	+	+	+	+
Impetigo-like eruptions				
Pruritic eczematous lesions	+	+	+	+
Severe progressive ulceration of lower extremities	+	+	+	+
Hair				
Low posterior hairline	+	–	–	–
Neurologic				
Central Nervous System				
Developmental delay	+	–	+	+
Hematology				
Neutropenia	+	–	–	–
Lymphopenia	+	+	–	+
Thrombocytopenia	+	–	–	–
Anemia	+	–	–	–

mo, months; yrs: years.

patient succumbed to multiorgan failure and sepsis. Initial PCR testing showed an Epstein-Barr virus copy number of 599, which rose to 665 000 after the DLBCL diagnosis.

Patients P3.1 and P3.2 had recurrent otitis, leading to a perforated tympanic membrane in P3.1. Both patients developed bronchiectasis, with P3.2 undergoing a lobectomy due to recurrent pneumonia.

Neurological Findings

Three patients (P1, P3.1, P3.2) had neurodevelopmental delays. Patient P2 had good academic performance and no active

Figure 2. Abnormal clinical phenotypes in prolidase deficiency. Patients exhibit various facial dysmorphisms. In P1, these include a prominent forehead, hypertelorism, ptosis, ocular proptosis, exophthalmos, upslanting palpebral fissures, a small nose, a low nasal root, and a slender upper lip. In P2, observed features include a prominent forehead, down slanting palpebral fissures, a low nasal root, and a beaked nose.

neurological symptoms until age 27, at which point she was monitored for epileptic seizures and altered consciousness, possibly related to lymphoma involvement.

Gastrointestinal Findings

Patient P1 experienced chronic diarrhea, failure to thrive, and abdominal distension beginning at 15 days of age. She required recurrent albumin infusions and enteral nutrition due to growth retardation. The patient was hospitalized multiple times for recurrent infections and malnutrition. At 5 months of age, physical examination revealed ascites and hepatosplenomegaly. Patient P3.2 also had a history of chronic diarrhea in the first 2 years of life. Three patients (P1, P2, P3.1) presented with hepatosplenomegaly.

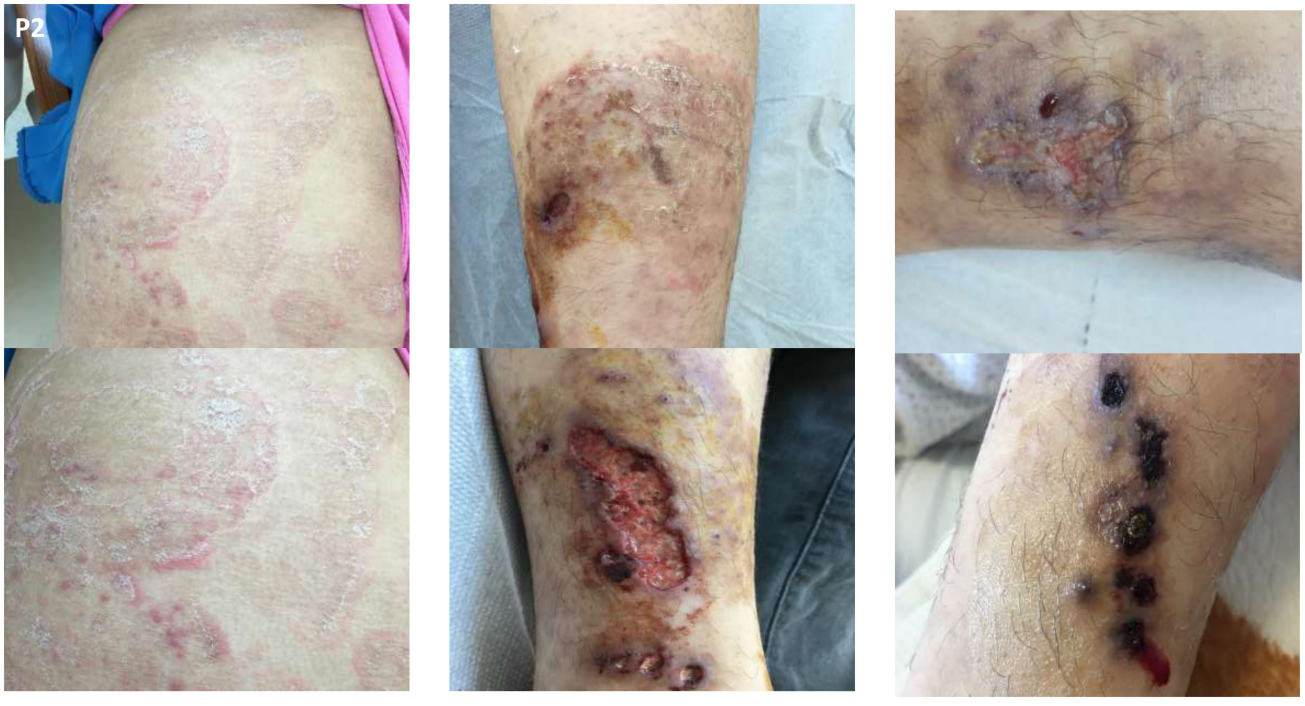

Figure 3. Clinical phenotypes of patients with PEPD deficiency, highlighting specific features. Skin lesions commonly observed in prolidase deficiency, particularly on the anterior surface of the tibia (P2). Left panel: Circinate, erythematous, scaly patches with active borders (potassium hydroxide mount positive). Middle panel: A scaly patch in the pretibial area that has ulcerated. The borders remain active, resembling tinea corporis. A punch biopsy from the ulcer border revealed fungal hyphae. Right panel: Hemorrhagic blisters at the pretibial site that progressed into deep ulcers. Multiple ulcers developed in the same area during follow-up.

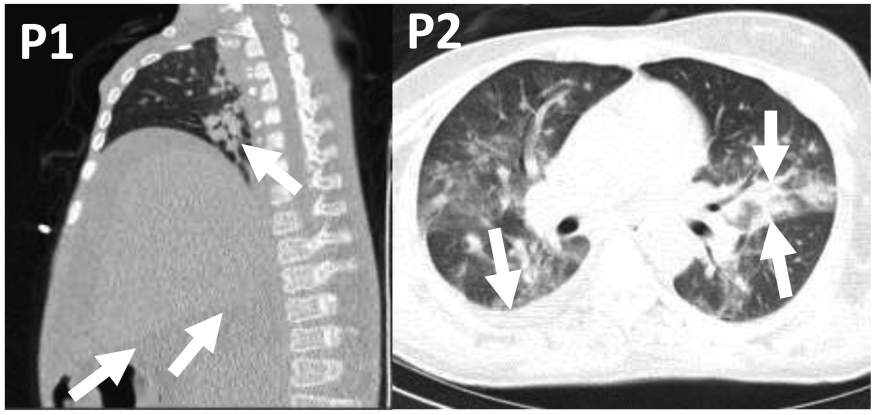

Figure 4. Radiological findings of patients with PEPD deficiency. Hepatosplenomegaly and atelectatic lung areas in P1. Diffuse ground-glass opacities during Pneumocystis jirovecii pneumonia in P2, with more prominent pleural effusion in the right lung. White arrows indicate the abnormalities.

Hematologic Manifestations

Patient P1 had thrombocytopenia, requiring repeated platelet transfusions. Patient P2 developed lymphoma during follow-up. Patients P3.1 and P3.2 had mild thrombocytopenia, which remained asymptomatic.

Metabolic Manifestations

Prolidase enzyme activity was measured in P1 and P2. Control prolidase enzyme activity was 381 ± 0.17 U/L, while patient values were lower at 12 ± 0.13 U/L and 110 ± 0.53 U/L, respectively, confirming the genetic diagnosis.

Genetic Analysis

Three biallelic variants were identified in the 3 families. Consanguinity was present in 3 patients, and the parents of patient P2 were from the same village. The following mutations were found: P1 had a c.220del, p.Ala74ArgfsTer56 frameshift variant; P2 had a missense variant, c.796A>G, p.Thr266Ala; P3.1 and P3.2 shared a c.1359_1361del, p.Glu453del in-frame variant.

Laboratory Assessment of Prolidase Deficiency

Detailed immunological evaluations are provided in Table 2. One patient (12.5%) had low serum IgG levels before starting immunoglobulin replacement. Serum IgA levels were low in 1 patient (25%), and serum IgM levels were low in 2 patients (50%). IgE levels were elevated in 2 patients (50%). Lymphopenia was observed in 3 patients (75%). Detailed analysis of lymphocyte subgroups showed a disrupted CD4/CD8 ratio in all patients, with a more pronounced imbalance in P1 and P2. Additionally, P1 and P2 had reduced naive T cells and recent thymic emigrants, suggesting combined immune deficiency at diagnosis. $CD19^+$ B cell levels were also low in P1 and P2. Additional immunological findings are outlined in Table 2.[14]

DISCUSSION

Prolidase deficiency is a rare inherited disorder that, while primarily presenting with dermatological manifestations, is not often considered in the differential diagnosis, necessitating thorough diagnostic evaluations.

The clinical diagnosis of prolidase deficiency can be established in a proband with characteristic clinical findings, including the presence of imidodipeptiduria or reduced prolidase enzyme activity. A molecular diagnosis can be confirmed in individuals with suggestive clinical features through the identification of biallelic pathogenic variants in the *PEPD* gene via molecular genetic testing.

In this cohort, initial differential diagnoses for affected individuals included immune dysregulation and multisystem inflammatory syndrome in children,[16] However, the diagnosis of prolidase deficiency was confirmed in all 4 patients by identifying pathogenic mutations in the *PEPD* gene. Additionally, 2 patients exhibited reduced prolidase enzyme activity, further supporting the diagnosis.

The clinical manifestations of prolidase deficiency are usually evident in the early years of life; however, some patients have been reported to present with resistant cutaneous lesions in adulthood. There are cases of asymptomatic patients reported during pubertal and adult ages (11 and 29 years old).[17-19] One (P2) of the patients presented clinically with significant post-pubertal skin lesions despite no significant medical history or history of infection. The course of their condition was severe, ultimately leading to complications secondary to malignancy. Another patient (P1) had a severe clinical course starting from the neonatal period, while symptoms in the other patient (P2) began in late childhood and adolescence. As reported in the literature, the severity and age of onset of the disease exhibit a wide spectrum.[20,21]

Dysmorphic facial features have been reported at high frequencies among patients with prolidase deficiency. In this cohort, all patients displayed similar dysmorphic features, though in 1 patient (P2), these were less pronounced. These features may not always become prominent until adulthood.[17,18,22,23]

Recurrent infections, especially pneumonia, are important components of prolidase deficiency and are commonly reported in diagnosed patients, causing the main reason for morbidity in affected individuals.[20] On the other hand, findings related to autoimmunity can be observed in patients, including systemic lupus and thyroiditis. However, in the 4 patients, autoantibodies were negative, and clinical autoimmunity was not detected.[18]

Table 2. Detailed Immunological Features of PEPD-Deficient Patients

Parameters (age at evaluation)	P1 (2 yrs)	P1 (5 yrs)	P2 (28 yrs)	P3.1 (13 yrs)	P3.2 (22 yrs)	Reference values* (2-5 yrs)	Reference values* (5-10 yrs)	Reference values* (10-16 yrs)	Reference values* (> 16 yrs)
ANC/mm^3	2300	**1400**	1900	2260	3000	1500-8500	1500-8500	1500-8500	1800-8000
ALC/mm^3	**200**	**900**	**700**	1880	**1300**	1703-6738	1803-5636	1403-4742	1400-7100
Eo/mm^3	0	60	100	50	0	0-500	0-500	0-500	0-500
Serum immunoglobulins									
IgG (mg/dl)	n.d.	1337	**568**	2374	1507	604-1941	746-1804	835-2694	913-1884
IgM (mg/dl)	n.d.	111	**31**	146	**81**	71-235	78-261	47-484	88-322
IgA (mg/dl)	n.d.	229	**37**	321	**418**	52-317	57-282	62-433	139-378
IgE (IU/ml)	**1568**	**1294**	6	**1458**	80	< 100	< 100	< 100	< 100
Specific antibody titers									
Anti-Hbs (IU/L)	n.d.	**> 10**	**< 10**	**< 10**	n.d.	> 10	> 10	> 10	> 10
Lymphocyte subsets									
CD3$^+$ T cells (%)	93.10	**92.01**	**50.6**	79	**89.57**	58-81	55-86	58-86	64-85
CD3$^+$ T cells (#)	186	**828**	**354**	1485	1164	1200-4706	971-3685	1032-3303	998-5625
CD3$^+$4$^+$ T cells (%)	18.58	37.01	**18.8**	35	33.66	24-52	23-49	27-47	32-58
CD3$^+$4$^+$ T cells (#)	37.2	**330**	**132**	658	**438**	458-2755	445-1918	505-1778	673-3110
CD3$^+$8$^+$ T cells (%)	75.79	**54.46**	29.4	**42**	**53.79**	12-36	17-47	17-39	14-39
CD3$^+$8$^+$ T cells (#)	151.6	490	**206**	790	699	165-1878	379-2084	381-1312	238-1570
CD19$^+$ B cells (%)	1.51	**1.82**	**2.4**	16	7.41	8-29	7-20	5-22	3-16
CD19$^+$ B cells (#)	3	**16**	**17**	301	96	205-1341	122-755	94-793	87-541
CD16$^+$56$^+$ NK cells (%)	3.59	**3.7**	**36.2**	5	10.61	4-22	4-29	2-27	5-25
CD16$^+$56$^+$ NK cells (#)	7.2	**33**	253	**44**	138	88-1393	105-1107	94-1175	91-766
T cell subsets (%)									
CD4$^+$CD45RA$^+$CCR7$^+$	n.d.	**0.52**	**1.26**	n.d.	n.d.	47-87	38-75	29-67	14-66
CD4$^+$CD45RA$^-$CCR7$^+$	n.d.	**0.36**	23.74	n.d.	n.d.	9-40	14-49	24-51	22-67
CD4$^+$CD45RA$^-$CCR7$^-$	n.d.	**97.63**	**73.25**	n.d.	n.d.	1-11	2-16	4-24	3-25
CD4$^+$CD45RA$^+$CCR7$^-$	n.d.	1.5	1.75	n.d.	n.d.	0.1-41	0.2-44	0.3-26	0.3-45
CD8$^+$CD45RA$^+$CCR7$^+$	**4.91**	**3.19**	4.65	n.d.	n.d.	19-83	18-62	11-61	4-68
CD8$^+$CD45RA$^-$CCR7$^+$	1.66	3.66	20.64	n.d.	n.d.	0.9-9	0.3-8	2-14	1-28
CD8$^+$CD45RA$^-$CCR7$^-$	35.55	55.34	29.98	n.d.	n.d.	4-43	2-33	6-38	0.7-40
CD8$^+$CD45RA$^+$CCR7$^-$	**57.88**	**37.82**	**44.73**	n.d.	n.d.	6-56	15-62	6-66	4-66
CD4$^+$CD45RA$^+$CD31$^+$	n.d.	**0.62**	**1.52**	n.d.	25.65	48-77	39-66	25-64	10-57
B cell subsets (%)									
CD19$^+$CD27$^-$IgD$^+$	66.76	45.41	38	n.d.	**87.8**	58-87	45-85	44-88	34-79
CD19$^+$CD27$^+$IgD$^+$	15	**3.78**	5.24	n.d.	**1.57**	5-21	4-24	2-22	5-32
CD19$^+$CD27$^+$IgD$^-$	8.53	15.68	**51.5**	n.d.	7.09	3-32	7-31	3-29	6-35
CD21^{low}CD38^{low}	11.4	7.57	11.2	n.d.	1.3	1-13	2-15	1-15	1-14

Abnormal values are shown as bold.
*Normal ranges were adopted from published normal national reference[14].
#, absolute value; ALC, absolute lymphocyte number; ANC, absolute neutrophil number; Eo, eosinophil number; CD4+ naïve T cells, CD4+CD45RA+CCR7+); central memory CD4+T cells, CD4+CD45RA−CCR7+; effector memory CD4+T cells, CD4+CD45RA−CCR7−; terminally differentiated effector memory CD4+T cells, CD4+CD45RA+CCR7−; recent thymic emigrants, (CD4+CD45RA+CD31+; CD8+ naive T cells, CD8+CD45RA+CCR7+; central memory CD8+T cells, CD8+CD45RA−CCR7+; effector memory CD8+T cells, CD8+CD45RA−CCR7−; terminally differentiated effector memory CD8+T cells, CD8+CD45RA+CCR7−; naive mature B cells, CD19+CD27−IgD+; non-switched memory B cells, CD19+CD27+IgD+; switched memory B cells, CD19+CD27+IgD−.

The mutation found in the 2 siblings was previously reported in 1994.[21,24] These siblings presented with a classical phenotype characterized by recurrent infections, leg ulcers, and dysmorphic features. Similar to this cases' variants in exon 12 of the *PEPD* gene, like c.825del and c.826G>A, were identified in a patient with diarrhea.[25,26] The P1 variant was in exon 3, c.220del. The limited results in this study suggest that there is no clear genotype-phenotype relationship in prolidase deficiency.

In previously reported cases, a patient was reported to have developed squamous cell carcinoma from the leg lesion.[27] However, there is no evidence in the literature regarding the development of lymphoma. With increasing cases and published reports, malignancy may emerge as a notable feature.

Immunological evaluation of the patients revealed elevated levels of IgE in 2 patients, a finding consistent with previous reports that describe hypergammaglobulinemia and a HIES-like phenotype have been reported in these patients.[28-30] Interestingly, severe changes in lymphocyte subsets of patients were obtained. P1 and P2 presented with leaky combined immunodeficiency-like phenotype, suggesting that the disease

can manifest with more severe immunological characteristics. Previously, detailed immunological evaluations of twins, including the assessment of lymphocyte subsets, revealed normal T, B, and NK lymphocytes, including $CD4^+/CD8^+$ T lymphocyte ratios.[31] In contrast, $CD4^+/CD8^+$ ratios were altered in all the patients in this study, and decreased B cells were observed in 2 patients. These results suggest that changes in lymphocyte subtypes can occur in prolidase deficiency. However, it should be noted that the immunophenotypic changes observed in P1 and P2 may be secondarily influenced by the presence of splenomegaly during the evaluation.

CONCLUSION

In conclusion, by presenting 4 new cases of prolidase deficiency, the diverse clinical manifestations of this condition were highlighted in the context of inborn errors of immunity. Beyond the well-known dermatological findings, recurrent opportunistic infections, gastrointestinal involvement, malignancy, and flow cytometry findings suggestive of combined immunodeficiency emphasize that prolidase deficiency may be underdiagnosed. The findings significantly contribute to the literature by facilitating earlier recognition and treatment of affected patients.

Availability of Data and Material: The data generated during the study are included in this published article.

Ethics Committee Approval: This study was approved by Ethics Committee of MarmaraUniversity Faculty of Medicine (approval number: 2013-0200; date: 28.06.2016).

Informed Consent: Written informed consent was obtained from the all patients' legal guardians who agreed to take part in the study.

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Beyond Dermatological Findings: Multisystem Involvement in Prolidase Deficiency – A Report of Four Cases

Patient 1 (P1). A female, born on April 8, 2012, from a consanguineous union, first presented at 15 days of age with clinical manifestations of diarrhea, failure to thrive, abdominal distension, malnutrition, hepatosplenomegaly, and ascites. She was hospitalized due to congenital cytomegalovirus (CMV) infection complicated by secondary nephrotic syndrome. Subsequent hospitalizations were required throughout the follow-up period for recurrent infections and malnutrition

During the follow-up period, the patient exhibited mental and motor developmental delays. Physical examination identified significant findings, including hepatosplenomegaly, ascites, growth and developmental delays with an absence of speech, cachexia, exophthalmos, dysmorphic nails, dental caries, digital clubbing, and pruritic lesions on the arms. Due to persistent hypoalbuminemia, she required multiple albumin infusions. Genetic testing revealed a homozygous mutation in the *PEPD* gene.

At 10 years of age, she was admitted to the hospital with a severe infection that progressed to sepsis, ultimately resulting in multi-organ failure and death.

Patient 2. A female, born on March 10, 1989, presented to the dermatology clinic at age 27 with swelling and erythema in the fingers of her right hand and foot. Prior to this presentation, she had no significant medical history or medication use. Four months after the dermatology visit, she sought medical attention due to prolonged fever, anorexia, and weight loss. Investigations for prolonged fever revealed 6-7 black-crusted skin lesions in a linear pattern on the distal right tibia, bilateral ground-glass opacities in the lungs with a positive Rhinovirus PCR in the nasopharyngeal swab. Her primary differential diagnosis included cutaneous nocardiosis and mycosis. The skin biopsy pathology revealed numerous fungal hyphae identified in the follicular epithelium and lumen within keratin in the epidermis. The patient received antifungal therapy for the lesions

Flow cytometry of lymphocyte subgroups showed isolated CD4 lymphopenia and she was diagnosed with idiopathic CD4 lymphopenia with negative HIV-RNA at the age of 28. Therefore, immunoglobulin replacement therapy was initiated along with antifungal and antiviral prophylaxis. At age 29, a hematological consultation prompted a positron emission tomography-computed tomography (PET-CT), which demonstrated widespread FDG uptake in the lymph nodes. Excisional biopsy confirmed high-grade B-cell lymphoma, and chemotherapy was initiated.

During the first cycle of the chemotherapy, she developed neurological symptoms, including fixed gaze and seizures, led to a cranial magnetic resonance imaging, which demonstrated central nervous system involvement. Due to worsening symptoms such as drowsiness and lack of responsiveness to stimuli, she was intubated and transferred to the intensive care unit. She passed away at the age of 29 due to sepsis, respiratory distress syndrome, and multi-organ dysfunction.

Patient 3.1 (P3.1). The patient, born on 19.07.2010, whose parents are consanguineous, began experiencing frequent otitis and pneumonia at 6 months old. Upon presentation, she exhibited hypertelorism, downward slanting palpebral fissures, a flat nasal bridge, a thin upper lip, an elongated thin nasal bridge, dysmorphic features, developmental delay inconsistent with age, and neuromotor retardation. At age 3, she developed widespread skin lesions on both legs, characterized by erythematous, crusted, erosive, and occasionally ulcerated areas. A skin biopsy showed a histological pattern consistent with subacute-chronic spongiotic dermatitis, reported as findings in line with atopic dermatitis. A previous biopsy from an external center had resulted in a diagnosis of bullous impetigo. During follow-up, at age of 6, she developed bilateral tympanic membrane perforation secondary to recurrent otitis and bronchiectasis following multiple pneumonia. Genetic analysis was performed at the age of 9 and revealed a homozygous *PEPD* mutation, and she has since been followed up with a diagnosis of prolidase deficiency.

Patient 3.2 (P3.2). This patient is a sibling of P3.1 and born on 12.11.2000. She had a history of recurrent bronchiolitis and diarrhea starting in infancy. At 6 months old, she was hospitalized due to bronchopneumonia, and had a history of recurrent otitis media, and developed bronchiectasis and bilateral tympanic membrane perforation during follow-up. The patient underwent lobectomy due to bronchiectasis. Additionally, starting from the age of 5, there were dysmorphic features, mental motor retardation, and widespread erythematous, crusted, erosive, and occasionally ulcerated lesions on both legs. She had recurrent diarrhea until the age of 2 without etiology, but no episodes were reported after that age. Genetic analysis at the age of 20 revealed a homozygous *PEPD* mutation, and she has since been followed up with a diagnosis of prolidase deficiency.