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Quantitative analysis of the natural history of prolidase deficiency: description of 17 families and systematic review of published cases

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Conflicts of Interest

No conflicts of interest to declare

Ethics declaration

For all patients with identifiable data, informed written consent for publication (including for pictures when applicable) was obtained and archived by the authors. In case of minors or adults unable to consent by themselves, the consent was obtained from their legal guardian. Each protocol was approved by its respective Institutional Review Board or follows local IRB or ethics committee regulations. There is no central IRB for this study. The main IRBs for this study are the National Institutes of Health IRB and Robert Debré University Hospital IRB. In other cases, ethics approval was obtained or waived by local regulations (Art L. 1121-1 of the French Public Health Code, Art. 53 of the French Data Protection Act, Recital 26 EU GDPR; Centro Hospitalar Universitário do Porto; Ethics Committee of the Medical Faculty, Justus Liebig Universität Giessen; Hospital Álvaro Cunqueiro; Seattle Children's Hospital IRB; UCSF Benioff Children's Hospital Oakland IRB; University of Chicago Biological Science Section IRB; University of Florida).

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Abstract

Purpose: Prolidase deficiency is a rare inborn error of metabolism causing ulcers and other skin disorders, splenomegaly, developmental delay and recurrent infections. Most of the literature is constituted of isolated case reports. We aim to provide a quantitative description of the natural history of the condition by describing 19 affected individuals and reviewing the literature.

Methods: Nineteen patients were phenotyped per local institutional procedures. A systematic review following PRISMA criteria identified 132 articles describing 161 patients. Main outcome analyses were performed for manifestation frequency, diagnostic delay, overall survival, symptom-free survival, and ulcer-free survival.

Results: Our cohort presented a wide variability of severity. Autoimmune disorders were found in 6/19, including Crohn's disease, systemic lupus erythematosus and arthritis. Another immune finding was hemophagocytic lymphohistiocytosis (HLH).

Half of published patients were symptomatic by age 4 and had a delayed diagnosis (mean delay 11.6 years). Ulcers were present initially in only 30% of cases, with a median age of onset at 12 years old.

Conclusion: Prolidase deficiency has a broad range of manifestations. Symptoms at onset may be nonspecific, likely contributing to the diagnostic delay. Testing for this disorder should be considered in any child with unexplained autoimmunity, lower extremity ulcers, splenomegaly, or HLH.

Introduction

Prolidase deficiency (OMIM 170100) is a rare autosomal recessive disorder caused by pathogenic variants in the *PEPD* gene, encoding for prolidase (EC 3.4.13.9)[1]. Prolidase acts as a dipeptidase, cleaving the imide bond present when either proline or hydroxyproline is in the C-terminal position of a dipeptide, thus forming an imidodipeptide; its highest activity is against glycylproline[2,3]. The enzyme is a homodimer and requires manganese as a cofactor[3,4]. The overall prevalence of prolidase deficiency is unknown. Data from the urine newborn screening program in Quebec suggested a prevalence of 1:1,235,000 (based on 2 cases overall)[5]. However, prolidase deficiency may be more commonly found in some populations: a carrier frequency of 1:21 was found in the Druze population in northern Israel[6], and a founder variant has been identified in the Amish settlements in Geauga County, Ohio[7].

Since its first description by Goodman and colleagues in 1968[8], over 160 different cases of prolidase deficiency have been described. Typical features include chronic ulceration (mostly of the lower limbs), telangiectasias, dysmorphic features, developmental delay, splenomegaly, recurrent infections and hematological abnormalities[9]. More recently, associations with chronic lung disease[10] and with systemic lupus erythematosus[11] have been identified as well. Amino acid analysis show a distinctive pattern, with massive elevation of imidodipeptides in urine, usually most strikingly glycylproline[12]; proline, and to a lesser proportion hydroxyproline, are elevated after hydrolysis of the sample[13]. Lesser elevations of imidodipeptides can also be detected in plasma, if the analysis is sensible

enough for detection. Diagnosis can be confirmed by prolidase enzymatic activity assay (in erythrocytes, leukocytes, or fibroblasts, usually performed in a research setting) or, more clinically available molecular analysis of the *PEPD* gene.

To this day, most of the literature on prolidase deficiency includes case reports or small cases series; only a few groups have published cohorts of more than 5 patients, given the rarity of the condition. To expand the understanding on this condition, we first aim to describe a cohort of 19 individuals with prolidase deficiency, seen by a network of collaborators in Europe and the United States. Then, we performed a systematic review of the literature in order to gain insight on the natural history of the condition.

Materials and Methods

Patient information

Data for each patient was collected systematically for demographic information, ancestry, consanguinity, clinical features, diagnosis and attempted treatments. Evaluations and investigations were performed as per local procedures for each center. For patient 17, rapid genome screening with targeted phenotype-driven analysis was performed at 7 weeks of life as part of California's "Project Baby Bear"; the methods have been previously published[14].

Review of the literature

When possible, principles outlined in the PRISMA statement[15] of the EQUATOR Network were applied. A literature search was conducted on PubMed, using the keywords "prolidase", "*PEPD*", "iminodipeptiduria" or "imidodipeptiduria", for case reports and case series written before September 2020, date of our last search (Figure 1). Data obtained from experiments conducted on patient's tissues were also included. No review protocol was registered beforehand. One author was responsible of performing the review. Articles or abstracts written in English, French, Spanish, Italian, Portuguese and German were included and translated, if needed. Reference lists from each article were scanned to identify further references, including journals not indexed in MEDLINE. Information about each patient was then compiled, including manifestations, diagnostic information and treatment attempts. As missing data was expected to be non-random, in order to reduce publication bias, only manifestations clearly stated were included as part of the phenotype, and manifestations not listed were imputed to be absent[16]. Data that could not be assigned to a specific patient or family were excluded from statistical analysis. In cases of inconsistencies between reports, the outcome reported in the majority of reports was used; in cases of equality, the most recent reported outcome prevailed, or in case of numerical values, a mean was used. Only families with either biochemically confirmed (imidodipeptiduria or low prolidase enzymatic activity), molecularly proven (*PEPD* pathogenic variants) or with a clear statement stipulating the diagnosis was confirmed were included. Patients described more than once were identified through cross-referencing or by matching key clinical data (e.g., clinical history and *PEPD* variants) in articles with shared authors.

Statistical analysis

Statistical analysis was performed on both literature data and patients described here. As patients 12 (Süßmuth et al.[17]) and 14 (Besio et al. patient 1[18]) were previously described, their most recent information was included only once.

When only a qualitative age assessment was available, it was converted into a numerical estimate (early infancy, 1 year old; infancy, 2 years old; early childhood, 8 years old; childhood, 11 years old; adolescence, 18 years old); if an age range was given, the mean was used. To perform genotype-phenotype analyses, cases were classified based on apparent homozygosity or compound heterozygosity for 1) missense variants or in-frame small deletions / duplications, 2) loss-of-function (LoF) variants, and 3) splicing variants; compound heterozygotes for two types of variants (e.g., missense and LoF) were excluded. Enzymatic activity values were converted as percentages of the reported normal for the assay and averaged together.

Counts and percentages were obtained for categorical variables; mean, median, range and standard deviation were obtained for continuous variables. For survival, Kaplan-Meier analyses were performed using GraphPad Prism 8.3; patients were censored at the age of their last known follow-up. Other analyses were performed using R version 4.0.2. Diagnostic delay was calculated as the difference between the age at diagnosis and the age at onset of symptoms. If the age at diagnosis was not explicitly stated, it was estimated to be the age at the time of report. For diagnostic delay analyses, only the longest diagnostic delay in each family was included. Linear regression between age of onset and age at diagnosis was performed, after confirming normality (Shapiro-Wilk test). The regression slope obtained was compared to a theoretical slope of 1 (age of onset = age of diagnosis) using Student's *t*-test. For associations between genotypes and main reported manifestations, Fisher's exact test was used, whereas for enzymatic activity and main reported manifestations, unpaired *t*-tests or Mann-Whitney tests were used, depending on distribution (Shapiro-Wilk test) and variance (*F*-test); a Bonferroni correction was applied. Hierarchical clustering analysis of main manifestations and major organ-systems affected was performed using Ward clustering algorithm. For all analyses, adjusted *p*-values were considered significant only if ≤ 0.05 (two-sided).

Results

Clinical description of our cohort

Nineteen patients from 17 different families are described in Tables 1 and S1. Aged between 1 and 34 years old at last assessment, their first manifestations occurred between the prenatal period and late childhood, presenting with various combinations of symptoms including skin lesions, neurologic and developmental anomalies, recurrent infections and hematologic anomalies. Most (15/19, 79%) presented dysmorphic features, most commonly affecting the eyes and nose (Figure 2, A-G). The majority (17/19, 89%) presented with dermatologic manifestations (Figure 2, H-R), but only 11/19 (58%) presented ulcers, the finding most commonly associated with prolidase deficiency in the literature. Other commonly described features included developmental delay (13/19, 68%), splenomegaly

(13/19, 68%, 4 also presenting with hepatomegaly), anemia (12/19, 63%), thrombocytopenia (10/19, 53%), gastrointestinal involvement (7/19, 37%) including 2 patients with Crohn's (or Crohn's-like) disease and chronic pulmonary disease in 5/19 (26%) including bronchiectasis (2/19), interstitial lung disease (2/19) or asthma (1/19). Various immunological anomalies were also described, including hyperimmunoglobulinemia E in 5/19 (26%), systemic lupus erythematosus features in 2/19 (11%) with one patient fulfilling ACR criteria[19] (positive ANA, arthritis, thrombocytopenia and positive anti-Smith antibodies), other autoimmune arthritis in 2/19 (11%) including juvenile idiopathic arthritis and psoriatic arthritis, and hemophagocytic lymphohistiocytosis (HLH) in one patient (patient 14), as defined by HLH-2004 criteria[20]. Other striking features found in only one patient are progressive cirrhosis (patient 18) and gangrene requiring amputation of toes and some fingers (patient 19).

Literature review

The primary search yielded a total of 841 results (Figure 1). A total of 128 articles describing patients with prolidase deficiency have been identified, spanning from 1968 to 2020. Through reference review of these articles, 10 other articles were identified. Three articles could not be retrieved, and three others were excluded for language reasons. All other 132 articles were reviewed for patient information (Table S2)[1-3,7,8,10-13,17,18,21-141]. One hundred sixty-one (161) different patients were identified through this review. Two articles were reviewed but contained data not traceable to specific individuals, and one article did not provide any type of diagnostic confirmation for three patients; they were excluded from further analyses. Including the data from the 19 subjects described before, a total of 178 patients were included in final analyses. Demographic data and clinical manifestations of this population can be found in Table 2; data on described variants and key biochemical parameters can be found in Tables S3-4.

Review of clinical manifestations (present cohort and literature data)

Manifestations at initial presentation were available for 139 patients (79%) (Figure 3A). More than one initial manifestation could be found in many patients. The most frequent presenting features were ulcers (42/139, 30%) and other skin manifestations (36/139, 26%), as well as frequent infections (29/139, 21%) and developmental involvement (24/139, 17%). On the other hand, 5 patients were reported as asymptomatic at the time of last follow-up (mean follow-up 13.5 years, range 0.3-29); they were diagnosed either because of a positive urinary newborn screening or because of an affected family member.

A summary of the main clinical manifestations reported throughout the course of the disease can be found in Figure 3B (more details in Table 2 and S5). The most frequently reported manifestations are dermatologic (84%), including ulcers (62%) with scarring (30%), various rashes (28%), telangiectasia or poikiloderma (22%) and eczema (16%). Dysmorphic features were found in 67% of patients, most commonly hypertelorism (35%), proptosis (18%) and a saddle nose deformity (14%) or low nasal root (10%), sometimes with poliosis (11%), frontal bossing (8%), high palate (7%) and either micrognathia (7%) or prognathism (3%). Developmental anomalies were frequent (58%), ranging from mild to severe; the intelligence quotient (IQ) was reported in 18 patients, ranging from 30

and 90. Although gait problems have been reported (7%), most of them were associated with pain due to lower extremity ulcers; other reported neurologic abnormalities included seizures (3%) and neuropathy (2%). Hematologic abnormalities (39%) included anemia (30%) and thrombocytopenia (18%). Recurrent or severe infections were present in around half of patients (48%). Proven immunological anomalies were frequent (25%), including hyperimmunoglobulinemia E (hyper IgE) and other hyperimmunoglobulinemias as well as neutropenia. Reported musculoskeletal anomalies (34%) included various limb anomalies, often minor and affecting hands, feet and lower limbs, most often brachydactyly or deformities secondary to ulcers; arthritis or synovitis was reported in 5% of cases. Splenomegaly (45%) was more frequent than hepatomegaly (14%); some patients were reported with elevated transaminases (7%) or liver disease (5%). Autoimmune disorders were present in 27 individuals (15%), including systemic lupus erythematosus in 10 (6%), combined with rheumatoid arthritis in 3 other cases (2%); autoimmune gastroenteropathies in 5 (3%); autoantibodies were reported in 36 different cases (21%) including anti-nuclear and anti-dsDNA antibodies. Chronic pneumopathies were reported in 30 individuals (17%), including asthma in 13 (7%) but also more severe pulmonary involvement in 22 patients (12%), including bronchiectasis, interstitial lung disease or pulmonary hypertension. There were no clearly distinct clinical subgroups of patients identified following hierarchical cluster analysis (Figures S1-2). Treatment of manifestations varied widely (Table S2). They include combinations of skin grafting, antibiotics, proline or glycine and proline ointments, supplementation of proline, ascorbic acid or manganese, immunosuppressive agents, blood transfusions, plasmapheresis, hyperbaric oxygen therapy or hematopoietic stem cell transplants. Although some were promising in individual case reports, reported effects are mostly inconsistent.

Diagnostic delay

A total of 104 individuals were included in diagnostic delay calculations (Figure S3). The mean time to obtain a diagnosis was 11.6 years (SD 10.6, range 0 – 41.75); half of the cases took 8.5 years or more before confirming a diagnosis of prolidase deficiency. When age of diagnosis is plotted against age of onset (solid line, Figure 3C), the slope significantly differs (Student's t-test = 3.54, p-value = 6.06×10^{-4}) from the ideal situation where the diagnostic delay is 0, i.e., when age of onset equals age at diagnosis (dashed line, Figure 3C).

Survival analyses

With the available data, three different Kaplan-Meier curves were built for survival analysis: overall survival, symptom-free survival and ulcer-free survival (see Figure 3D-F).

A total of 20 cases (11%) were reported as deceased in the literature. Age of death for these individuals ranged from 3 months to 50 years old. Causes of death were reported in a few and included respiratory failure (4/20), infectious complications (3/20), fulminant hepatitis (1/20), cardiorenal amyloidosis (1/20) and post-operative (1/20) or post-hematopoietic stem cell transplant complications (1/20). The oldest reported living individual was 64 years old at the time of the report. Overall, 90% (95% CI 83-94%) of patients were alive by age 20, 88% (95% CI 81-93%) by age 30 and 82% (95% CI 70-90%) by age 40 years old (Figure 3D).

Data about the age of onset was available for a total of 124 patients; half of these patients developed symptoms by age 4, 90% had symptoms by age 14 and 95% by age 17 (Figure 3E). Only 5 patients remained asymptomatic at the time of publication of the reports. The mean age of follow-up for these asymptomatic patients is 13.5 years old (range: 0.3 – 29 years old). As for survival without ulcers, data from 74 reported cases where the age of onset of ulcers was known were included in the analysis, together with 58 patients without ulcers at the time of the last report. Median age of ulcer development in this cohort is 12 years old; almost 75% of patients will have developed ulcers by 18 years of age (Figure 3F).

Genotype-phenotype and enzymatic activity analyses

Genotype-phenotype analysis was performed by Kaplan-Meier analyses (Figure 3G and S4) as well as comparison between the three variant categories (missense, LoF, splicing) and main manifestations (Figure S5, Table S6). There was a significant difference between the three genotypic groups ($p = 2.38 \times 10^{-5}$ using Fisher's exact test and after Bonferroni correction; $p = 0.0025$ for Mantel-Cox test on Kaplan-Meier analysis). Pairwise differences between the missense and LoF groups as well as missense and splicing groups reached significance on Fisher's exact test (adjusted $p = 0.00015$ and $p = 0.003$, respectively), but not between the LoF and splicing groups (adjusted $p = 1$). On Mantel-Cox test with the Kaplan-Meier analysis, only the difference between missense and LoF reached significance ($p = 0.0013$); the difference was non-significant between missense and splicing groups ($p = 0.067$). Comparisons with other manifestations or symptom-free survival did not show any difference between the groups.

As for enzymatic activity, correlation with overall survival, symptom-free survival, ulcer-free survival or any of the main manifestations of prolidase deficiency, all lacked any significant difference between the groups (Figures S6-8 and Table S7).

Discussion

We described here one of the largest case series of prolidase deficiency with 19 affected individuals from 17 families. Together with quantitation of clinical characteristics found in 161 cases from the literature, we were able to provide a deeper understanding of the natural history of this condition.

Diagnostic delay for patients with prolidase deficiency is considerable; patients waited an average of 11.6 years before diagnosis, with more than half waiting 8 years or more. This is similar to other rare conditions, where diagnosis can take several years[142-144]. Prolidase deficiency is most often considered as part of the differential diagnosis of skin ulcers. However, only around a third of patients had ulcers at the time of presentation. It may take years between the initial presentation and the development of ulcers: at 4 years of age, half the patients presented with symptoms of prolidase deficiency, but only 15% of patients exhibited ulcers; half of all patients developed ulcers by age 12, with some patients never developing them. Another possible reason for this diagnostic delay is the lack of specificity of some of the presenting symptoms, such as various rashes, recurrent infections, organomegaly or developmental delay; the phenotype may remain nonspecific. When the possibility of an inborn error of metabolism is evoked, urine amino acids are generally

considered much later in the workup. Biochemical diagnosis can also be challenging. Imidodipeptide elevations may be mistaken for amino acid elevations[12]. Routine plasma amino acids are unlikely to detect any diagnostic abnormalities if not specifically screened for, although glycylproline has been detected in some cases[8,44,84,138]. The rise of mass spectrometry-based assays may complicate identification of iminodipeptiduria, as it requires specific monitoring for the corresponding ions and most commercially available kits for amino acid analysis do not include any imidodipeptide. Imidodipeptiduria can be detected as part of urinary newborn screening[45], but only a few jurisdictions offer it. These factors, combined with the general lack of awareness about this condition, may all contribute to diagnostic delay.

Interestingly, some degree of genotype-phenotype correlation exists in prolidase deficiency. Individuals with biallelic missense variants are less likely to develop ulcers than individuals with loss-of-function variants, and they develop them later. This finding was highly significant, despite conservative adjustment. This may have implications for counselling following molecular analysis for families and may further contribute to diagnostic delay in these individuals. There was no correlation found with other manifestations, and analyses of enzymatic activity were all non-significant; this may be due to the lack of data, to important differences in enzyme assay methodologies (even in the same tissue), or to other biological differences (e.g., variants affecting non-enzymatic activity).

There is growing evidence about predisposition to immune disorders in prolidase deficiency. In our cohort, 6 patients presented autoimmune disorders (including Crohn's disease, systemic lupus erythematosus or lupus-like disorder, psoriatic arthritis and juvenile idiopathic arthritis), and an additional patient presented isolated elevated ANA. Altogether, at least a fifth of patients in the literature presented some degree of autoimmunity. Other immunological anomalies are also present in a significant number of patients, including hyper IgE, neutropenia, and seldom hypergammaglobulinemia or hypocomplementemia. Recurrent infections were present in 13 patients in our cohort and in almost half of the total cohort. Although skin infections can be at least partially explained by the presence of ulcers, other frequent infections such as respiratory infections cannot be explained by this mechanism.

Another immune phenomenon for which the association with prolidase deficiency is described here is hemophagocytic lymphohistiocytosis (HLH). Patient 14 presented at age 8 an episode fulfilling HLH criteria[20]: fever, increase in her usual splenomegaly, pancytopenia (hemoglobin 68 g/L [6.8 g/dL], leukocytes 2.8 x 10^9 cells/L [2,800/μL] with 0.7 x 10^9 neutrophils/L [700/μL], platelets 20 x 10^9 cells/L [20 x 10^3/μL]), hypofibrinogenemia (0.3 g/L [30 mg/dL]), hyperferritinemia (up to 11,000 μg/L [11,000 ng/mL]), as well as evidence of hemophagocytosis on bone marrow aspiration. She had a positive Epstein-Barr Virus PCR. Initial treatment with intravenous immunoglobulins, corticoids and ganciclovir induced rapid improvement in her clinical status. The possibility of HLH was also evoked for patient 12 at 7 months of age, given 4 criteria were fulfilled: splenomegaly, cytopenia of 2 lineages (hemoglobin 70 g/L [7.0 g/dL], platelets 70 x 10^9 cells/L [70 x 10^3/μL]), hyperferritinemia (up to 8,842 μg/L [8,842 ng/mL]) and increased interleukin-2 levels (up to 3,127 U/mL); NK cell activity was however not consistent with HLH and

fibrinogen remained normal. He received treatment for two months, including cyclosporine and dexamethasone. Prolidase deficiency should be considered as part of the differential diagnosis of HLH. To address this, *PEPD* sequence analysis should be added to HLH gene panels. It can also be addressed in the workup at the same time as lysinuric protein intolerance (LPI, *SLC7A7*), another inherited metabolic disease predisposing to HLH, as the investigation of this condition also involves urine amino acid analysis.[145]. LPI was indeed the differential that was searched for when investigations were initiated for patient 4 in the setting of persisting and isolated hepatosplenomegaly. Patient 4 also fulfilled three HLH criteria, namely splenomegaly, cytopenia (hemoglobin 84 g/L [8.4 g/dL], platelets 70 x 10^9 cells/L [70 x 10^3/μL]) and hyperferritinemia (up to 1,041 μg/L [1,041 ng/mL]).

Taken together, these observations suggest a role of prolidase in immunity as a whole. Hypotheses involving the complement system and chemotaxis have been proposed by various authors[78,117]. Prolidase has been associated to the regulation of transforming growth factor β (TGFβ)[146], hypoxia-induced factor 1α (HIF-1α)[147] and epidermal growth factor receptor (EGFR)[148], either through its imidodipeptidase activity (for TGFβ and HIF-1α) or through protein-protein interactions (for EGFR)[149]. Prolidase is released from damaged cells and can activate AKT, ERK and STAT3 through EGFR signaling, suggesting a role in tissue injury and inflammation[148]. Furthermore, derivatized imidodipeptides such as alaninyl-L-boroproline have been shown to affect T-cell proliferation *in vitro* by inhibiting dipeptidyl peptidase-4 (DPP4), an important peptidase responsible for cleaving N-terminal Xaa-Pro in polypeptides[150]. Some authors have suggested that the accumulation of imidodipeptides in prolidase deficiency may similarly cause inhibition of DPP4 and other peptidases[151,152]. This may in turn affect the regulation of numerous biologically active peptides containing Xaa-Pro N-terminal motifs, including several proteins and cytokines involved in immunity and in the HLH cytokine storm[151,153]. Interestingly, data from the International Mouse Phenotyping Consortium (www.mousephenotype.org)[154] demonstrates several NK and T cell abnormalities in *Pepd* knockout mouse models, which may provide some insights into potential mechanisms of HLH predisposition in prolidase deficiency. Further characterization of the role of prolidase in immune regulation would be warranted to gain a better understanding of these phenomena.

We also reported here three patients with genital abnormalities, which were not known from analysis of the previously reported literature. Morphological abnormalities are not infrequent in prolidase deficiency: dysmorphic features are found in more than half of the patients, and several musculoskeletal abnormalities have been described. These findings, together with the presence of developmental delay in many affected individuals, raise questions about the role of prolidase in embryonic and early life development. An embryonic role of prolidase has been shown in one study, where prolidase-deficient mice developed cardiac hypertrophy[155]; however, cardiovascular abnormalities have only been reported in a few affected individuals (5/178, 3%), none of which presented cardiomyopathy. Developmental abnormalities of the cerebral cortex[156] and of the bones[18] have been reported in postnatal mouse models of prolidase deficiency. Hypotheses about the role of prolidase in degradation and recycling of collagen, a peptide rich in proline, as well as hypotheses regarding a brain deficiency in proline also remain to be elucidated[157].

Even if this analysis allows for a deeper understanding of prolidase deficiency, it does not replace a prospective natural history study. The different methods of assessment and evaluation of affected individuals introduce variability in the data. Details on diagnostic criteria for some conditions (e.g., SLE) were not always available, and it is not possible to exclude that some manifestations could be explained by another unrelated condition. Some inconsistencies have been found between different reports of a given patient. Our analysis method may cause some manifestations to be underreported, as a symptom not clearly stated as present was considered to be absent for statistical purposes; however, this also likely prevented overestimation of the prevalence of other manifestations. Some manifestations may be overrepresented, as they are more likely to lead to investigation of prolidase deficiency and, subsequently, publication. Conversely, mild forms of the disorder or unusual cases with severe but non-classical manifestations are likely to be overlooked and underreported, particularly cases without ulcers.

In conclusion, this meta-analysis style approach to the literature, combined with the description of 19 new cases of prolidase deficiency, allowed the available data on an ultra-rare disorder to be collected in a systematized manner. It illustrates the wide variability in clinical presentation, including the various and sometimes nonspecific initial manifestations, and the need for an increased awareness to enable early diagnosis. It also allowed the identification of the key clinical patterns and main complications, which in turn can inform clinical care of affected individuals with early identification of complications. These findings may help the development of natural history studies, which are primordial to future therapeutic developments for this still poorly treatable condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FR, MSDM, CRF and MS drafted the manuscript. JFB, MB, EB, AC, BC, CC, JLDD, AD, DD, AFF, MTG, CG, TG, SSH, JL, CTL, LM, EM, AM, IM, AKO, NP, DQ, AS, AMS, ARS, SLS, KS, JT, CRF and MS have seen the subjects described here and/or provided original data about the subjects. The systematic review and statistical analyses were devised and performed by FR and CRF. All authors have reviewed the final version of this manuscript before submission. Special thanks to Dr. Nataliya Tkachenko, who was part of the medical team evaluating patients 7 and 8.

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Data availability

Data used throughout this publication is available in the Supplementary Materials.

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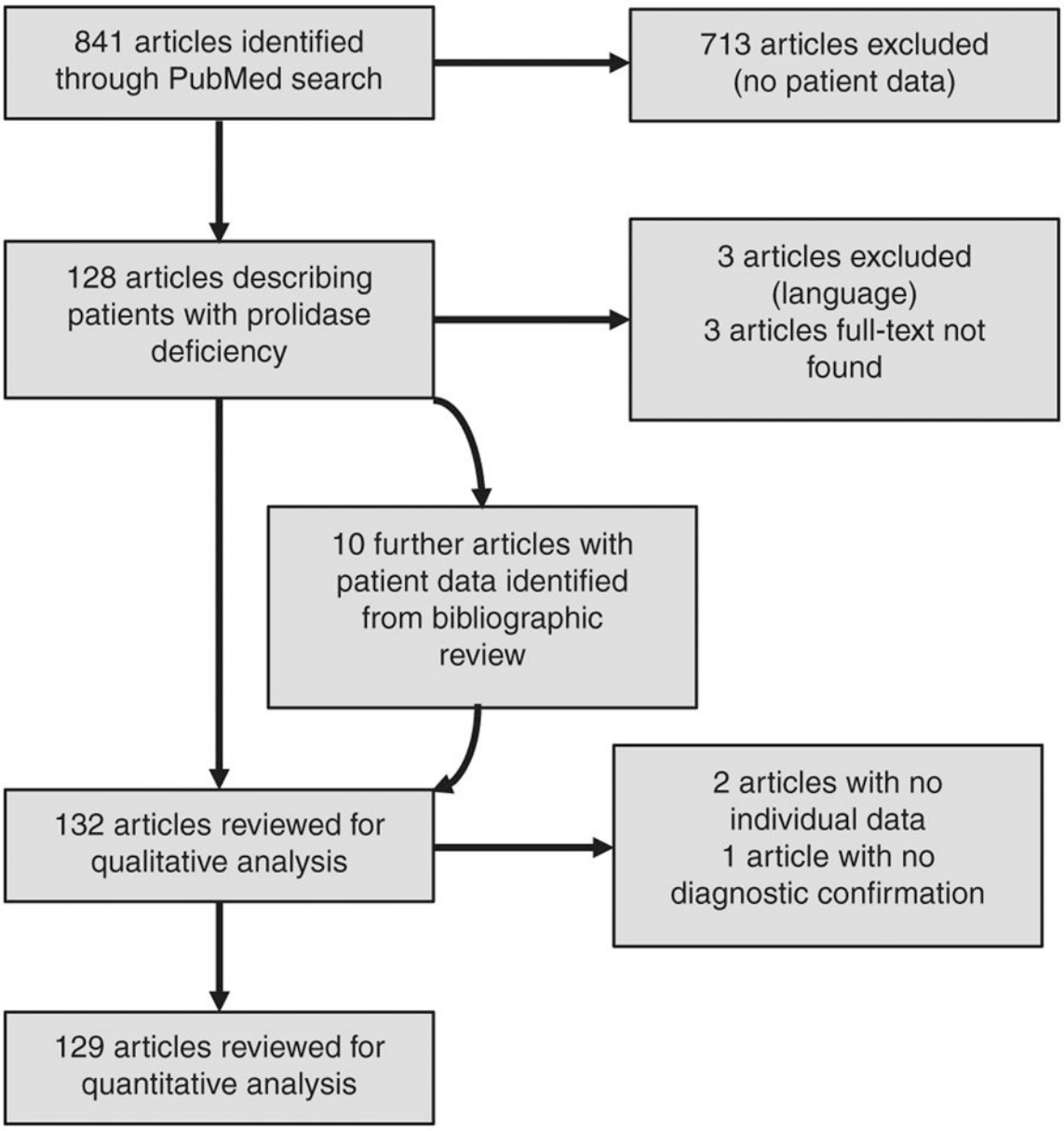

Figure 1:
Flow diagram of the systematic review of the literature.

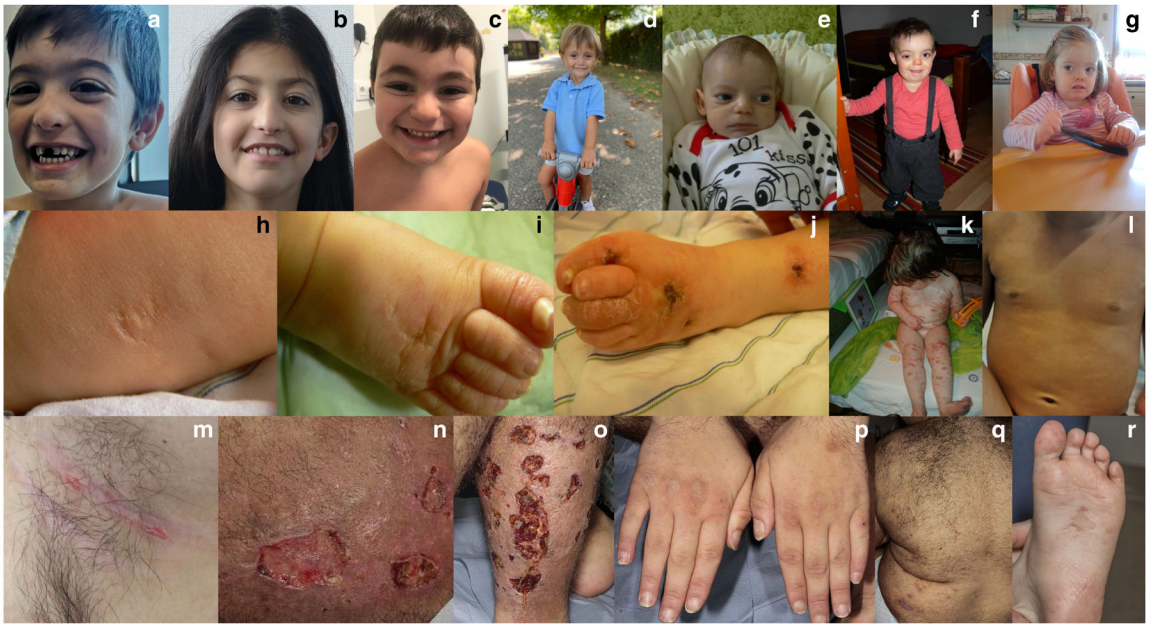

Figure 2: Clinical characteristics of prolidase deficiency.

A-G: Facial features of patients 7, 8, 10, 11, 12 (E & F) and 14, respectively, including in some a high and/or prominent forehead, hypertelorism, epicanthal folds, ptosis, a low nasal root and/or hypoplastic alae nasi. H-J: Evolution of a typical ulcer, from onset (H) to final stages (J) (patient 12). K-R: Dermatologic manifestations, including pityriasis rubra pilaris (K, patient 14), pigmentary changes (L, patient 8), ulcers of variable severity (M, patient 9; N-O, patient 5), hyperkeratosis and distal erythema (P, patient 5), hirsutism with folliculitis (Q, patient 5) and telangiectasias (R, patient 14).

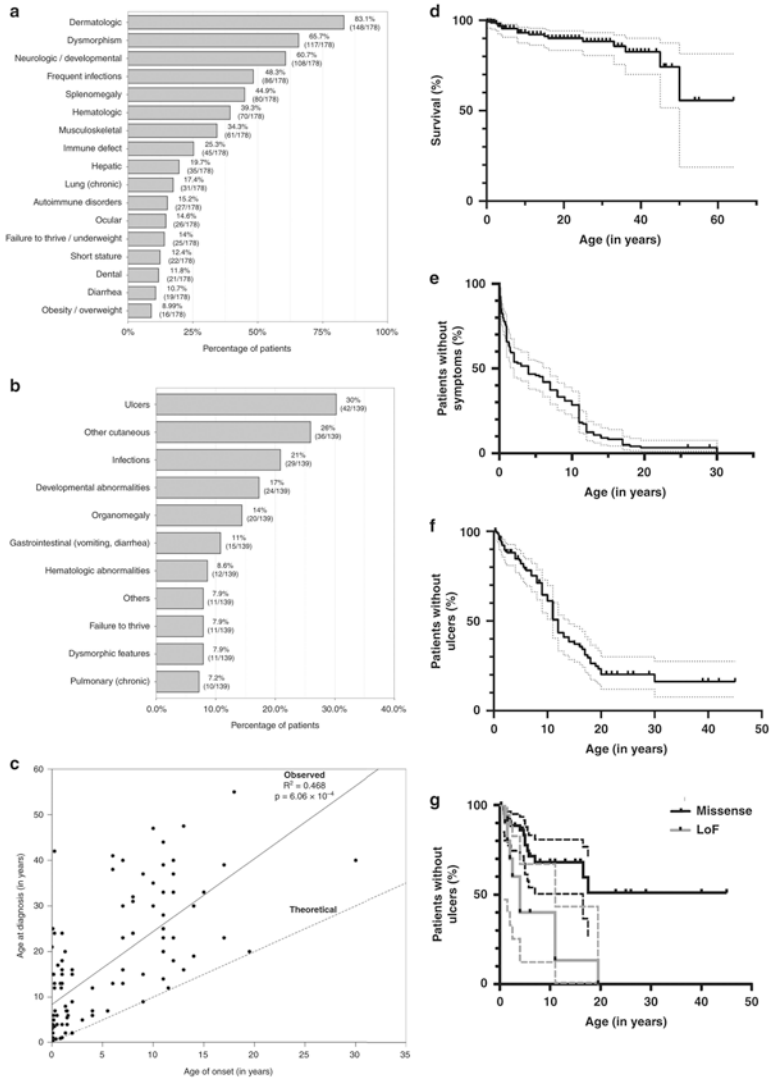

Figure 3: Clinical manifestations (A-B), age of onset (C) and survival (D-G) analyses.

A-B: Main clinical manifestations reported at onset (A) and overall (B). C: Linear regression model, including the observed slope (solid line) and the theoretical slope (dashed line) of diagnostic delay. D-G: Overall survival (D), symptom-free survival (E), and ulcer-free survival for the entire cohort (F) and for missense and loss-of-function variants (G).

Table 1:

Clinical description of patients from our cohort

Family / Patient	Gender	Age at last assessment (y)	Age of onset (y)	Ulcers	Other skin	Dysmorphic features	Chronic respiratory	Hepato/ splenomegaly	GI involvement	Hematologic anomalies[a]	Recurrent infections	Immune anomalies[b]	Developmental delay	Imidodipeptiduria	*PEPD* variants
I/1	M	8	0.3	−	+	+	−	−/−	+	−	−	−	+	+	N/A
II/2	F	13	0.3	−	+	+	+	−/+	+	−	+	L	+	+	N/A
II/3	M	18	4.0	−	+	−	+	−/+	+	−	+	A/L	+	+	N/A
III/4	F	2	0.4	+	+	−	−	+/+	−	A/T	+	H/L	+	+	+
IV/5	M	20	1.3	+	+	+	+	−/−	−	−	+	−	−	N/A	+
V/6	M	27	7.0	+	+	+	−	−/+	−	A	+	A	+	+	N/A
VI/7	F	7	0.5	−	+	+	−	−/+	−	A/T	+	−	+	+	+
VI/8	M	4	0.3	−	+	+	−	+/+	−	A/T	+	−	−	N/A	+
VII/9	F	25	N/A	+	+	−	−	−/+	−	A	+	H	+	+	+
VIII/10	M	5	N/A	−	−	+	−	−/−	−	−	−	−	+	+	+
IX/11	M	2.5	0.2	+	+	+	−	−/+	+	A/T	−	H	−	+	+
X/12	M	9	0	+	+	+	+	+/+	+	A/T	+	L/G	+	+	+
XI/13	M	6	1.5	+	+	+	−	−/+	+	A/T	+	L	+	N/A	+
XII/14	F	9	0	+	+	+	−	−/+	−	A/T	−	A/+	+[c]	+	+
XIII/15	M	4.5	PN	−	+	+	−	−/+	+	A/T	+	A	+	+	+
XIV/16	F	14	11	+	+	+	−	−/−	−	−	+	H	+	+	+
XV/17	F	1.25	0	−	−	+	−	−/−	−	−	−		−	+	+
XVI/18	F	34	0.25	+	+	+	+	+/+	−	A/T	+	H	−	+	+
XVII/19	F	4	0.5	+	+	−	−	−/−	−	A/T	−	−	−	N/A	+

Abbreviations: y: years; PN: prenatal; GI: gastrointestinal, N/A: not available.

[a] A: Anemia; T: thrombocytopenia.

[b] A: autoimmune disorder; G: hypogammaglobulinemia; H: hyper IgE; L: leukocyte abnormality; +: HLH.

[c] With regression

Table 2:

Demographic and clinical information from patients of the literature and our cohort

	n **(mean, range)**	**%**		***n***	**%**		***n***	**%**
Patients	178	-	**Dysmorphic features**	117	65.7%	**Musculoskeletal**	61	34.3%
Consanguinity	81	46.0%	Hypertelorism	62	34.8%	Hand / feet anomalies	24	13.5%
Deceased	20	11.4%	Proptosis	32	18.0%	Other limb anomalies	10	5.6%
Gender	167	93.8%	Saddle nose	25	14.0%	Osteopenia	13	7.3%
Female	85	50.9%	Low hairline	21	11.8%	Hypermobility	13	7.3%
Male	82	49.1%	Poliosis	19	10.7%	Arthritis	9	5.1%
Age (y)	(19.3, 0.3 – 64)	92.6%	Low nasal root	17	9.6%	**Hematologic**	70	39.3%
Age of onset (y)	(5.5, 0.0 – 30.0)	75.6%	Frontal bossing	15	8.4%	Anemia	53	29.8%
Diagnostic delay (y)	(11.7, 0.0 – 41.75)	57.4%	High / ogival palate	13	7.3%	Thrombocytopenia	32	18.0%
Diagnosis			Micrognathia	13	7.3%	Pancytopenia	9	5.1%
Imidodipeptiduria	124	69.7%	Lip dysmorphism	12	6.7%	**Immune**		
Low prolidase activity	93	52.2%	Ear dysmorphisms	9	5.1%	Frequent infections	86	48.3%
PEPD variants	96	53.9%	**Ocular anomalies**	26	14.6%	Auto-immune disease	22	12.4%
Growth parameters			**ENT / Dental**			Lupus (SLE)	10	5.6%
Failure to thrive	25	14.0%	Chronic sinusitis	16	9.0%	Rhupus	3	1.7%
Overweight / Obesity	16	9.0%	Dental anomalies	21	11.8%	Partial lupus	5	2.8%
Short stature	22	12.4%	**Thoracic**			Autoantibodies	36	20.2%
Microcephaly	9	5.1%	Chronic lung disease	22	12.4%	HyperIgE	9	5.1%
Dermatologic	148	83.1%	Asthma	13	7.3%	Other hyperIg	26	14.6%
Ulcers	111	62.4%	Digital clubbing	13	7.3%	Other immune	7	3.9%
Ulcer infections	30	16.9%	**Gastrointestinal**			**Endocrine**	8	4.5%
Scarring	53	29.3%	Hepatomegaly	24	13.5%	Delayed puberty	5	2.8%

	n **(mean, range)**	**%**		***n***	**%**		***n***	**%**
Rash	49	27.5%	Liver disease	8	4.5%			
Telangiectasias	39	21.9%	Elevated transaminases	12	6.7%			
Eczema	28	15.7%	Splenomegaly	80	44.9%			
Xerosis	23	12.9%	Diarrhea	19	10.7%			
Crusting	20	11.2%	Autoimmune gastroenteropathy	5	2.8%	Reported in ≤5%: tall stature, thin skin, purpura, Raynaud phenomenon, acrocyanosis, nail anomalies, craniosynostosis or other suture anomalies, narrow or large palpebral fissures, epicanthal folds, cleft lip/palate, prognathism, neck anomalies, hearing loss, thoracic cage anomalies, pulmonary embolism, cardiovascular anomalies, hypertension, jaundice, seizures, neuropathy, talipes, genu valgum, spina bifida, scoliosis, contractures, hemolysis, coagulation anomalies, psychiatric disorder		
Hyperkeratosis	17	9.6%	**Renal**	**14**	7.9%			
Pigmentary changes	16	9.0%	**Urogenital**	4	2.2%			
Edema	13	7.3%	**Neurologic**	108	60.7%			
Pruritus	13	7.3%	DD / ID / LD	104	58.4%			
Visible veins / Livedo	9	5.1%	Hypotonia	9	5.1%			

Abbreviations: *n*, number of individuals; %, percentage; y, years; ENT, ear, nose and throat; DD / ID / LD, developmental delay, intellectual disability, learning difficulties; SLE, systemic lupus erythematosus; hyperIgE, hyperimmunoglobulinemia E; hyperIg; hyperimmunoglobulinemia