Identification of a Homozygous PSTPIP1 Mutation in a Patient With a PAPA-Like Syndrome Responding to Canakinumab Treatment

Alexandra Geusau, MD; Nadine Mothes-Luksch, MD; Hesam Nahavandi, MD; Winfried F. Pickl, MD; Carol A. Wise, MD; Zahra Pourpak, MD; Elisabeth Ponweiser, Leopold Eckhart, PhD; Raute Sunder-Plassmann, MD

Background: Pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome (OMIM 604416) is a rare autosomal dominant inherited autoinflammatory syndrome characterized by pyogenic sterile arthritis and less frequently accompanied by pyoderma gangrenosum and acne. It is associated with dominant missense mutations in the proline-serine-threonine phosphatase–interacting protein 1 gene (PSTPIP1) located on chromosome 15. The patient was diagnosed as having features of a PAPA-like syndrome in which cutaneous manifestations, such as pyoderma gangrenosum and acne fulminans, predominated.

Observations: Sequencing of the PSTPIP1 gene was performed in the patient and his extended family. The patient’s DNA analysis revealed a homozygous nucleotide exchange c.773G>C in the PSTPIP1 gene, leading to the substitution of glycine 258 by alanine (p.Gly258Ala), a previously reported heterozygous polymorphism. Heterozygous changes were identified in both of the patient’s parents and in 7 other family members, all of whom were asymptomatic. The patient was treated with canakinumab, a human anti–interleukin 1β monoclonal antibody, which led to rapid remission of the symptoms.

Conclusions: To our knowledge, this is the first reported case of the resolution of dermatological symptoms associated with a PAPA-like syndrome using canakinumab treatment. Further study of the p.Gly258Ala variant is warranted to determine whether this mutation has a role in causing an apparently recessive cutaneous syndrome resembling PAPA syndrome.

Hereditary autoinflammatory disorders are a heterogeneous group of rare diseases that do not necessarily affect every member of a family carrying the disease-causing mutation. The establishment of the correct diagnosis may be difficult because the clinical presentation often mimics infection.\(^1\) For many syndromes, the genetic basis has not yet been characterized, and research is ongoing to elucidate the pathogenesis of these immune-mediated diseases. Interleukin 1 (IL-1) and inflammasome activation have a pivotal role in the perpetuation of inflammation and may serve as a target for therapy. Even in the absence of an identified genetic alteration, patients exhibiting clinical symptoms compatible with an autoinflammatory condition should be considered for this new generation of therapeutics that block IL-1. In addition, if a phenotype is indicative of one of these diseases, the threshold for genetic analysis should be low, and efforts to elucidate possible molecular alterations should be undertaken if the genetic defect is unknown. In the patient described herein, genetic analysis suggests the existence of a novel syndrome resembling pyogenic sterile arthritis, pyoderma gangrenosum (PG), and acne (PAPA) syndrome (OMIM 604416) with a recessive inheritance pattern and a dramatic treatment response to canakinumab, a human anti–IL-1β monoclonal antibody.

REPORT OF A CASE

A 22-year-old Iranian man was admitted to the Department of Dermatology, Medical University of Vienna, Vienna, Austria, in poor clinical condition in the autumn of 2006. He had a fever and was experiencing painful acne distributed predominantly on the trunk (Figure 1A), as well as extensive serpiginous ulcers on his left upper and lower leg with violaceous undermined borders covered with fibrinous material, a condition indicative of PG.
Inspection of his oral cavity revealed aphthous lesions on the buccal mucosa; candidiasis was present. Extended atrophic scars on the legs, buttocks, and back of the patient had originated from prior episodes of painful ulcers. The patient exhibited no inflammatory arthritis, joint mutilation, or clinical symptoms of inflammatory bowel disease, such as abdominal pain, cramping, or diarrhea. At his initial admission in 2006, a blood workup showed a highly elevated C-reactive protein level of 24 mg/dL (reference range, <1 mg/dL), a white blood cell count of 22,000/µL (reference range, 4,000-10,000/µL), and moderate hypochromic microcytic anemia (to convert C-reactive protein level to nanomoles per liter, multiply by 9.524; to convert white blood cell count to ×10⁹/L, multiply by 0.001). The differential white blood cell count demonstrated neutrophilia of 86% (reference range, 50%-75%) and relative lymphopenia of 7% (reference range, 25%-40%) (to convert neutrophilia and lymphopenia fractions to proportion of 1.0, multiply by 0.01).

Initially, serum immunoglobulin analysis revealed an elevated serum IgA level of 1080 mg/dL (reference range, 70-500 mg/dL), a normal IgG level of 1010 mg/dL (reference range, 700-1600 mg/dL), and a normal IgM level of 47 mg/dL (reference range, 40-280 mg/dL) (to convert IgA level and IgM level to milligrams per liter, multiply by 0.001; to convert IgG level to grams per liter, multiply by 0.01). Antinuclear antibodies and rheumatoid
factors were negative, as was serologic testing for hepatitis A, B, and C and human immunodeficiency virus. Immunophenotyping of whole blood cells was performed as previously reported. Increased CD64 of 31% (reference value, 20%) and elevated CD16 of 86% (reference value, <10%) expression indicated activation of granulocytes and monocytes, respectively. The CD4/CD8 ratio was normal (1.52), with reduced numbers of CD4+ (380/μL) and CD8+ (250/μL) T cells, indicating dysregulation within the T-cell compartment.

For lymphocyte proliferation assays, peripheral blood mononuclear cells (PBMCs) (×10^6 per well) were incubated with staphylococcal superantigen A (10 ng/mL), staphylococcal superantigen B (20 ng/mL), phytohemagglutinin (12.5 μg/mL), or phorbol myristate acetate (10−7 mol/L) (all from Sigma Chemicals) or with soluble CD3 monoclonal antibody (2 μg/mL; Ortho) or plain medium in 96-well round-bottom tissue culture plates for 72 hours. Tetanus toxoid (10 times flocculation per well) and purified protein derivative (2 μg/mL) (both from Statens Serum Institute) and medium only were incubated with PBMCs for 6 days. Subsequently, cells were pulsed with [methyl-3H] thymidine (1 μCi per well) for another 18 hours and were processed as previously described. Although the patient's PBMCs reacted robustly but significantly less than control PBMCs to phytohemagglutinin (34 vs 113 kilo counts per minute [kcpm]), weak proliferation was observed on stimulation with staphylococcal superantigen A (24 vs 91 kcpm) and staphylococcal superantigen B (5 vs 43 kcpm), the phorbol ester phorbol myristate acetate (12 vs 81 kcpm), and soluble CD3 monoclonal antibody (6 vs 19 kcpm). Moreover, in contrast to the control individual, significantly reduced recall responses to tetanus toxoid (4 vs 27 kcpm) and purified protein derivative (2 vs 78 kcpm) were observed in the patient. Oxidative burst of neutrophils was normal on phorbol myristate acetate stimulation and was reduced on N-formyl-methionyl-leucyl-phenylalanine stimulation. Phagocytosis of opsonized Escherichia coli particles was normal.

The patient reported that he had been experiencing skin ulcers and acne lesions since age 14 years. From early childhood, he had had recurrent episodes of arthralgia, painful joints, and fever that did not respond to the administration of high-dose antibiotics on the occasion of repeated hospitalizations. The patient is the eldest of 3 siblings, with his younger sister also occasionally experiencing acne-like lesions. The patient and his sisters are descendants of a consanguineous marriage (ie, his mother and father are double first cousins) (Figure 1C). The medical history and the coincidence of cystic acne and PG were indicative of PAPA syndrome, which belongs to the family of autoinflammatory disorders and is caused by mutations in the proline-serine-threonine phosphatase-interacting protein 1 gene (PSTPIP1) located on chromosome 15, encoding a protein also known as CD2-binding protein 1 (CD2BP1).

We performed sequence analysis of the PSTPIP1 gene in our patient. Four milliliters of blood was drawn from the patient after obtaining his informed consent for DNA analysis. In addition, DNA was isolated from 23 family members living in Iran, according to standard proce-
amyloid A levels (Figure 2C). He reported sickness, without fever or arthralgia. Within a few days following the first injection, his skin improved significantly, and the inflammatory variables decreased. Additional injections were administered according to the given schedule, and he remained well for the following 9 months, with a com-

Figure 2. Patient after cessation of systemic corticosteroids. A, Before treatment with canakinumab, the patient had dense folliculitis in the neck and face area. B, During treatment with canakinumab, complete remission is seen at the same sites after 9 months and 3 injections of canakinumab. C, Response patterns to canakinumab injections (150 mg subcutaneously) in the C-reactive protein (CRP) and serum amyloid A (SAA) levels are shown in the patient over time. The arrows indicate the canakinumab injections every 8 weeks (at days 57, 110, 167, and 223). At day 0 (6 days before the first injection of canakinumab), the CRP and SAA levels increased significantly. Note that the normal levels of CRP and SAA are less than 1 mg/dL and 0 to 6.4 mg/L, respectively (to convert C-reactive protein level to nanomoles per liter, multiply by 9.524). Within a few days after the first injection, the CRP and SAA levels dropped, accompanied by significant clinical improvement. Subsequently, the curves seem to follow a decrescendo pattern associated with the injections.
The coexistence of severe and unusual episodic arthritis in addition to ulcerative skin lesions and cystic acne clustered in a few families was originally termed familial recurrent arthritis. In 1997, this syndromic triad was named PAPA syndrome.8 So far, 34 affected individuals from 5 families (2 in the United States, 1 in Italy, 1 in the Netherlands, and 1 in New Zealand) have been described worldwide.5,8,12 Pyogenic sterile arthritis resulting in joint mutilation is reportedly the most common clinical representation. However, PAPA syndrome manifestations vary among family members carrying the mutation and can lack the clinical characteristics such as PG or a late onset in life.11 So far, 4 genetic variations in PSTPIP1 (p.Ala230Thr, p.Glu250Gln, p.Glu250Lys, and p.Asp266Asn) have been described that lead to this rare autoinflammatory disorder with autosomal dominant inheritance, primarily affecting the joints and skin6,8,13,14 (http://fmf.igh.cnrs.fr/ISSAID/infevers/search.php?n =5). In contrast to the previously reported PSTPIP1 mutations that cause autosomal dominant inherited PAPA syndrome, we present evidence that a homozygous PSTPIP1 mutation (c.773G>C and p.Gly258Ala) may define a novel form of a recessively inherited PAPA-like syndrome.

To estimate the functional significance of the p.Gly258Ala mutation, we determined the degree of evolutionary conservation of the Gly258 residue by comparative analysis of PSTPIP sequences derived from the Ensembl genome database (http://www.ensembl.org/). Gly258 was found to be conserved, with a single exception, among all mammals investigated (eFigure). By contrast, Gly258 was not conserved in nonmammalian vertebrates (eFigure). Remarkably, none of the species investigated had an alanine residue at the amino acid position corresponding to Gly258. Together, these findings indicated an important role of Gly258 in maintaining the structural integrity of PSTPIP1 or in facilitating intermolecular interactions of PSTPIP1 in mammals. Previously, Nesterovitch et al13 identified a heterozygous p.Gly258Ala mutation in 1 of 14 patients with PG and suggested an effect of the mutation on the multimerization of the PSTPIP1 protein or its interaction with PTPN12. However, this hypothesis has not yet been tested experimentally. Further investigations are required to unravel the molecular pathogenic mechanism of the PSTPIP1 p.Gly258Ala mutation.

Immunological investigations during the first episode of our patient’s disease course documented at our clinic revealed reduced numbers of CD4+ and CD8+ T cells, indicating dysregulation within the T-cell compartment. Proliferative responses to mitogens of PBMCs revealed a lower response compared with the control, without recall responses to tetanus toxoid and purified protein derivative. In addition, the patient was initially seen with very low B-lymphocyte numbers and with elevated serum IgA levels; their relevance to the clinical manifestation is unclear. Cortesio et al16 reported significantly impaired chemotaxis in macrophages from 2 patients with PAPA syndrome, whereas T-cell migration was not compromised.

Hypogammaglobulinemia and elevated tumor necrosis factor (TNF) serum levels, which were unrelated to the pathologic findings in our patient, have previously been reported in a patient with PAPA syndrome who improved after substitution of immunoglobulins.17 Cytokine production by PBMCs was studied in 2 patients with PAPA syndrome and showed that these patients produced more inflammatory cytokines on lipopolysaccharide stimulation, in particular IL-1β and IL-1α.11 The finding that elevated IL-1 levels lead to lymphopenia had already been observed in the 1990s, when laboratory animals were infused with a series of purified acute-phase proteins in an attempt to better understand the pathomechanisms involved in sepsis.18 One of the mechanisms accounting for this phenomenon might be a central nervous system mode of action of IL-1β, leading to stimulation of both the sympathetic nervous system and the hypothalamic-pituitary axis.19 That catecholamines have the tendency to suppress T-lymphocyte activation by lectins, such as concanavalin A, is well established20,21 and might involve signaling via β-adrenergic receptors involving the p38 mitogen–activated protein kinase pathway.22,23 Besides the antiproliferative effects, catecholamines might also favor T-cell apoptosis by increasing T-cell Fas ligand levels, potentially explaining the clinically evident states of lymphopenia observed in our patient.22

Considering the clinical presentation of the patient and his relatives, the effect of p.Gly258Ala seems to be less pronounced than or different from other mutations causing PAPA syndrome. In 7 of 9 heterozygous relatives, the amount of intact PSTPIP1 protein seems to be sufficient to compensate for the variant protein to prevent the development of PAPA-like syndrome. However, 2 of his mother’s siblings carrying this mutation had a history of acne, with one of them seen with active acne lesions on the back. Until now, the p.Gly258Ala substitution had been described as a polymorphism (rs34240327) because it was detected in heterozygous form with an allelic frequency of 1.4% (no homozygous p.258Ala carriers) in a collective of apparently healthy individuals (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi ?rs=34240327). If only the altered protein is present in homozygous carriers of p.Gly258Ala, the dysregulation of proinflammatory pathways leads to a novel phenotype of PAPA syndrome that mainly affects the skin (ie, ulceration and acne fulminans); joint involvement is mild, without mutilation. Notably, Nesterovitch et al13 recently described a patient with PG carrying a heterozygous p.Gly258Ala mutation and having a history of mild arthralgia and acne. In light of the minor effect of this variant on our patient’s heterozygous relatives, we speculate that the individual described by Nesterovitch et al...
was also experiencing a PAPA-like syndrome. Therefore, the heterozygous p.Gly258Ala mutation could represent the basis for a variant PAPA syndrome that becomes clinically manifest only in the presence of genetic modifiers (such as a second p.Gly258Ala allele in our patient). Another potential trigger might be microbial pathogens that lead to the activation of proinflammatory cytokines via lipopolysaccharides, initiating an immunological response and an increase in cytokine production, which in turn leads to disease flares. Our patient’s medical history bears this out; the symptoms were exacerbated when he contracted an additional infection. The required extra trigger factor may also be the reason why in oligosymptomatic patients PAPA syndrome can remain undiagnosed for a long time.

The ex vivo experiments performed in 2 patients with PAPA syndrome revealed an increase in the secretion of inflammatory cytokines by PBMCs (ie, IL-1α, IL-1β, and TNF) on lipopolysaccharide stimulation. These data provided by Schellevis et al11 and the present understanding about the pathogenesis of PAPA syndrome offer new treatment options in patients with this condition. For instance, subcutaneous injection of the IL-1 receptor antagonist anakinra resulted in significant improvement.23,24 Another option includes therapies targeting IL-1β by the long-acting fully human anti–IL-1β monoclonal antibody canakinumab, which is approved for the treatment of cryopyrin-associated periodic syndromes.25 The present study represents the first documented instance of a patient experiencing PAPA-like syndrome treated with canakinumab, which induced a rapid and sustained clinical response. According to the literature, and in accord with what is expected for PG, the symptoms of PAPA syndrome respond well to high dosages of corticosteroids,25 with PG being the most recalcitrant of the diseases.26 One patient with hypogammaglobulinemia and high TNF serum levels improved after intra-articular corticosteroid administration and monthly intravenous gamma globulin (400 mg/kg) therapy.27 Targeted therapies with anti–TNF blocker (ie, infliximab) showed excellent response, whereas the anti–TNF blocker etanercept did not provide significant clinical benefit in the treatment of PG in the same patient.25 However, etanercept was effective in a patient with predominant joint involvement.6

In summary, the results of our genetic analysis suggest the existence of a novel PAPA-like syndrome with a recessive inheritance pattern. Because PAPA and PAPA-like syndromes seem to vary in the expression of clinical phenotypes, many cases may remain undiagnosed. Furthermore, the pathogenesis of these syndromes as described herein is supported by the dramatic response in our patient to treatment with canakinumab.

Accepted for Publication: August 17, 2012.

Correspondence: Alexandra Geusau, MD, Division of Immunology, Allergy, and Infectious Diseases, Department of Dermatology, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria (alexandra.geusau@medunivien.ac.at).

Author Contributions: All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Geusau and Sunder-Plassmann. Acquisition of data: Geusau, Mothes-Luksch, Nahavandi, Pickl, Wise, Pourpak, Ponweiser, and Sunder-Plassmann. Analysis and interpretation of data: Geusau, Pickl, Ponweiser, and Sunder-Plassmann. Drafting of the manuscript: Geusau, Wise, Eckhart, and Sunder-Plassmann. Critical revision of the manuscript for important intellectual content: Geusau, Wise, Eckhart, and Sunder-Plassmann.

Conflict of Interest Disclosures: None reported.

Additional Contributions: Ebrahim Radinia, MD, and Leyla Sedighipour, MD, assisted with the study, Mahdokht Mossalaei and Petra Jurkowitsch provided technical help, and Tina Breckwoldt, PhD, revised the manuscript.

Online-Only Material: An eTable and eFigure are available at http://www.jamaderm.com.

REFERENCES


14. Edscre AF, Kaplan DL, Abdou NL. Pyogenic arthritis, pyoderma gangrenosum, c


### Skin Pores in Persian Medical Textbooks

“Skin pores” as an informal term is used for the outlet of “pilosebaceous units and sweat-producing glands.” However, “traditional Persian medicine,” as one of the branches of complementary medicine with roots that go back earlier than 8000 BC, has fully described “skin pores/pores” with the special term of “masams.” This rich school of traditional medicine believes that masams play an important role in the appearance of some symptoms and diseases and uses them in the management of many disorders.2,3

Masams are very small natural pores or openings, especially in the skin surface near the hair follicles, and also in other body organs (eg, the eyelids, eyes, stomach, uterus, placenta, muscles, bones, joints). Except for the eyelid’s masams, which are considered to be the same as skin masams, they act as a pathway for exchange of materials in other body organs. These pores are divided into 2 groups, perceptible (visible) and imperceptible (conceptual or functional), and various functions are associated with them.2,3

Persian medicine believes that respiration is one of the main responsibilities of skin and is performed basically by masams.2 Masams are also known as entrance pathways of materials, such as wind, steam, and foreign substances, and cause differences in the effects of medications by their selective penetration. They also play an important role in the excretion of waste materials by producing sweat, sebum, and hair.2,3 The production of sweat is also used as a main treatment strategy for fevers.2 The role of masams in various conditions, such as opening, dilation, and porosity, and also opposite conditions, such as obstruction, closure, tightening, and condensation, has also been considered, because they cause differences in the penetration of materials2,3 and, directly or indirectly, mediate in the manifestation of some symptoms. Masams are also mainly considered in the diagnosis and treatment of some diseases. Symptoms and diseases, such as alopecia, underweight disorder, infertility, and some types of fever, are caused by “obstruction or tightening” of masams. Conversely, “dilation” of masams causes diaphoresis, increase in body metabolism, and removal of material from the body. Many disorders, such as diarrhea, various fevers, hemorrhoids, skin eruptions, amenorrhea, and obesity, are also treated according to these alternations of masams.2,3

It is consequential that long ago, great Persian physicians, without having advanced and modern instruments and only by observation of signs and symptoms, realized that specific factors (macroscopic, microscopic, and nanoscopic pores) are associated with the transpiration of materials. They used the term “masams” for all these factors (not only for pilosebaceous units and sweat glands) and diagnosed and treated many diseases almost perfectly with this method.

Fatemeh Alizadeh, MD
Fatemeh Moradi, MD

### Author Affiliations:
Traditional Iranian Medicine Research Association, Student's Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran.
Contact Dr Alizadeh at Traditional Iranian Medicine Research Association, Exceptional Talent Developmental Center of Tehran University of Medical Sciences, No. 56, Vaseel Street, Keshavarz Boulevard, Tehran 1417633114, Iran (resadayamfaraj@yahoo.com).