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SYNDROMIC HIDRADENITIS SUPPURATIVA: GENOTYPE-PHENOTYPE CORRELATION THROUGH WHOLE-EXOME SEQUENCING IN 10 UNRELATED PATIENTS

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INDICE

1 Hidradenitis suppurativa

1.1 Clinical features and pathogenesis of hidradenitis suppurativa

Hidradenitis suppurativa (HS) is a chronic, inflammatory systemic disease affecting the skin with inflammatory nodules, abscesses and fistulous tracts on the axillary, inguinal and mammary folds and on the anogenital areas. [Jemec, 2012]

Disease severity ranges from mild HS presenting with localized lesions to severe HS manifesting as multiple areas of inflammation, nodules and abscesses possibly forming plaques and interconnected fistulas, leading to hypertrophic scars. [Jemec, 2012] The prevalence of HS is around 1% in Western Europe, and, of note, the average interval from the self-reported onset of symptoms to diagnosis is approximately 7 years. [Saunte, 2015]

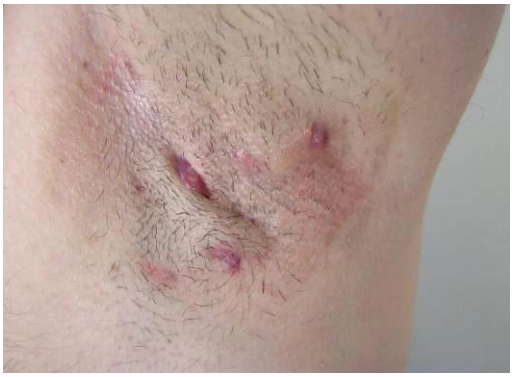
HS is a debilitating disease interfering with many activities of daily life. A recent study demonstrated that HS may have a greater impact on quality of life (QoL) than psoriasis and other chronic medical conditions. [Kolli, 2020]

The pathogenesis of HS is complex, resulting from the interaction between genetic factors, host-specific aspects (ie, metabolic syndrome and an abnormal innate and/or adaptive immune response), and environmental influences, such as bacterial microbiomes and cigarette smoking. [Micheletti, 2014] A family history of HS is present in almost 30% of cases, and some familial forms of HS have been suggested to be disorders with autosomal dominant inheritance. [Theut Riis, 2021] Genetic factors underlying familial HS have been reported only in few patients, with mutations of the genes encoding for the gamma-secretase complex being associated with some familial pedigrees and specific disease subsets. [Wang, 2021] In sporadic HS, the contribution of genetic factors is still an area of active research, and several studies, with the use of a genome-wide association study (GWAS) approach and whole-exome sequencing (WES), are underway to unravel the genetics of HS and have identified other genes

possibly associated with HS, such as fibroblast growth factor-receptor 2 (*FGFR2*) gene. [Higgins, 2017]

Furthermore, an innate immunity dysfunction leading to autoinflammation has recently been reported to play a crucial role, [Lima, 2016; Marzano, 2017] with overexpression of proinflammatory cytokines such as interleukin (IL)-1β and IL-17, and tumour necrosis factor (TNF)-α both in the lesional skin and in the serum of patients. [Marzano, 2017]

***Figure 1**. Fistulous tracts in the axillary cavity of a patient with hidradenitis suppurativa*



1.2 Syndromic hidradenitis suppurativa

In a minority of patients, HS may occur in the context of rare syndromes with other immune-mediated inflammatory diseases or inherited conditions, presenting as "syndromic" HS. [Gasparic, 2017] The definition of syndromic HS is still evolving, as there is a wide range of monogenic and polygenic conditions increasingly associated with the HS-lesional response pattern, thus complicating the diagnosis and classification of these complex conditions.

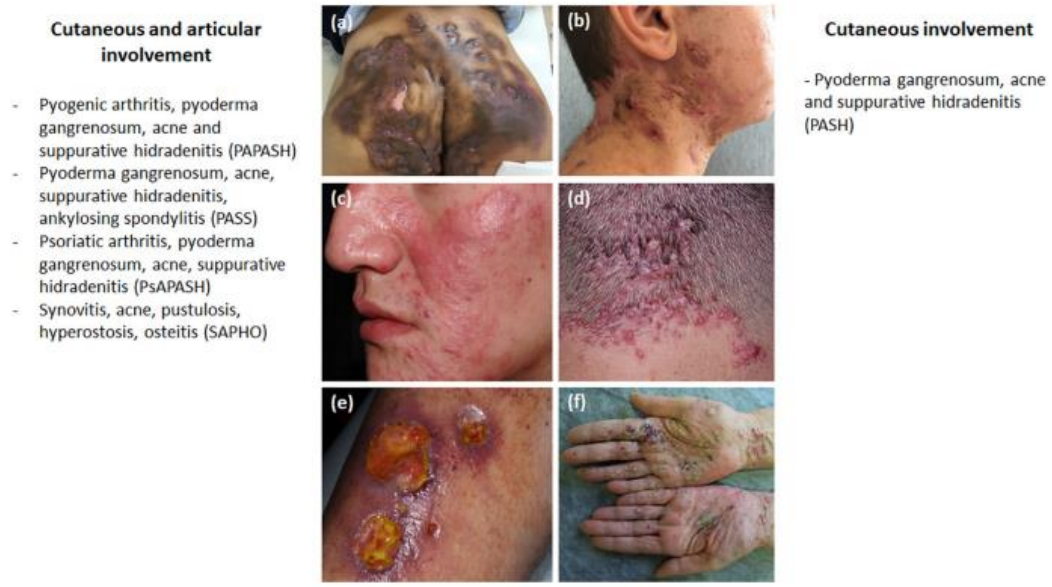
Three subtypes of syndromic HS have been suggested by Gasparic and coworkers [Gasparic, 2017]: syndromes with known genetic abnormalities, syndromes characterized by follicular plugging or structural defects, and syndromes with possible autoinflammatory pathogenesis.

***Figure 2**. Spectrum of conditions associated with hidradenitis suppurativa*

Classification	Syndrome	Involved genes or chromosomal alterations
Group 1	Keratisis-ichthyosis-deafness syndrome (KIDS)	*GJB2*
	Down syndrome	Trisomy chromosome 21
	Dowling Degos disease	*KRT5, POFUT1, POGLUT1, PSENEN*
Group 2	Follicular occlusion syndrome	Yet unknown
Group 3	Pyoderma gangrenosum, acne, suppurative hidradenitis (PASH)	*MEFV, NOD2, NLRP3, IL1RN, PSTPIP1, PSMB8, NCSTN*
	Pyogenic arthritis, pyoderma gangrenosum, acne, suppurative hidradenitis (PAPASH)	*PSTPIP1*
	Psoriatic arthritis, pyoderma gangrenosum, acne, suppurative hidradenitis (PsaPASH)	Yet unknown
	Pyoderma gangrenosum, acne, suppurative hidradenitis, ankylosing spondylitis (PASS)	Yet unknown
	Familial Mediterranean fever (FMF)	*MEFV*
	Synovitis, acne, pustulosis, hyperostosis, osteitis (SAPHO)	*LPIN2, NOD2, PSTPIP2, IL1RN*

Adapted from Gasparic et al.[10]
GJB2, gap junction beta-2 protein; *IL1RN*, interleukin-1 receptor antagonist; *KRT5*, keratin 5; *LPIN2*, lipin 2; *MEFV*, Mediterranean fever; *NCSTN*, nicastrin; *NOD2*, nucleotide-binding oligomerization domain-containing protein 2; *NLRP3*, NOD-, LRR-, and pyrin domain-containing protein 3; *POFUT1*, protein O-fucosyltransferase 1; *POGLUT1*, protein O-glucosyltrans-ferase 1; *PSENEN*, presenilin enhancer; *PSMB8*, proteasome subunit beta type-8; *PSTPIP1*, proline-serine-threonine phosphatase interacting protein 1; *PSTPIP2*, proline-serine-threonine phosphatase interacting protein 2.

***Figure 3**. Clinical images of patients with syndromic hidradenitis suppurativa*



1.3 Follicular occlusion syndrome

In 1956, Pillsbury and coworkers defined the follicular occlusion (FO) triad as a coexistence of acne, hidradenitis suppurativa and dissecting cellulitis of the scalp (DCS). Pilonidal sinus was subsequently added to form the definition of the FO tetrad; however, both entities may be referred to as the follicular occlusion syndrome (FOS). [Vasanth, 2014]

FO triad has been reported in association with keratitis ichthyosis deafness (KID) syndrome. Other reports used the terms FO triad and tetrad to describe incomplete

syndromes or even single features, for example HS, and fall outside the definition of the syndrome. [Vasanth, 2014]

A male and Afro-American preponderance has been found. Patients had a median age of 33 years. Lesions were located at predilection sites. Familial occurrence of HS, acne and pilonidal sinus was described in two cases. [Vasanth, 2014]

The reports on treatment are limited and incomplete. Tetracyclines were at least partially effective. Isotretinoin was reported effective in one and local antiseptics in two cases. Pharmacotherapy combined with surgery has been suggested. [Vasanth, 2014]

1.4 Bazex–Dupré–Christol syndrome

Bazex–Dupré–Christol syndrome (BDCS) is an X-linked congenital disorder, mostly involving the hair follicle. The syndrome is characterized by hypotrichosis, follicular atrophoderma, hypohidrosis and the development of basal cell neoplasms. Two mentions of BDCS were associated with HS; however, the reports are vague and provide no details. The association between BDCS and syndromic HS appears tenuous. [AlSabbagh, 2018]

1.5 Dowling-Degos Disease

Dowling-Degos disease (DDD, or reticular pigmented anomaly of the flexures) is a rare autosomal dominant genodermatosis, classically characterized by acquired reticular hyperpigmentation in flexural sites. Other features include perioral acneiform pitted scars and comedo-like lesions. DDD is caused by loss-of-function mutations in different genes, including keratin 5 (*KRT5*), protein O-fucosyltransferase 1 (*POFUT1*), and protein O-glucosyltrans-ferase 1 (*POGLUT1*), leading to different clinical phenotypes (generalized, flexural, and acantholytic forms). Histologically DDD shows a characteristic pattern of moderate follicular hyperkeratosis and plugging. [Stephan, 2021]

The clinical association of HS and DDD has been reported in various families and case series, with a female preponderance. It has been considered by some authors to be a specific disease subtype rather than a simple clinical overlap. [Stephan, 2021] The differential diagnosis of HS/DDD with secondary post-inflammatory hyperpigmentation is based on typical clinical, dermatoscopic and histological aspects. In published series, patients with HS/DDD commonly present with a family history of HS, a follicular lesional pattern, and atypical involvement of the trunk and nape. Familial co-occurrence of the DDD and HS has been described in three of four male cases. Standard treatments of HS reportedly provide unsatisfactory results. [Stephan, 2021]

Patients with the clinical HS/DDD phenotype may carry mutations in the genes encoding the gamma-secretase complex *PSENEN* and *NCSTN*, resulting in decreased Notch signaling. Mutations of DDD-associated genes like *POFUT1* have been also reported in the HS-DDD phenotype. This may also lead to impaired Notch signaling, a crucial pathway regulating the interfollicular and follicular epithelial differentiation. [Stephan, 2021]

1.6 Down syndrome

The possible association of HS with Down syndrome was first proposed in 1977. [Lam, 2020] Later, it was suggested that the association may be attributed to abnormal amyloid precursor protein (APP) expression. [Blok, 2014] The gene encoding for APP is located on chromosome 21 and APP promotes keratinocyte activity, which could play a role in follicular plugging. [Blok, 2014] Furthermore, APP and Notch receptors are competitive substrates for gamma secretase. A decrease in Notch receptor processing could be speculated to be the cause of impaired Notch signalling and, consequently, HS. [Blok, 2014]

A female preponderance and an onset of HS in the teens is reported. Obesity was described in four cases and, being associated with both HS and DS, may provide another link to co-occurrence.

Patients were most frequently treated with tetracyclines and flucloxacillin. Surgery was also used; however, the reports are vague regarding the effects of treatments. [Lam, 2020]

1.7 KID syndrome

Keratitis, ichthyosis and deafness (KID) syndrome is a rare congenital ectodermal dysplastic disorder, associated with mutations in the connexin-26 and connexin-30 genes, GJB2 and GJB6. The resulting hyperproliferative state of epidermis may contribute to follicular plugging and the onset of HS. [Bettoli, 2021] The syndrome is characterized by the presence of vascularizing keratitis, reticulated palmoplantar hyperkeratosis, neurosensory deafness, erythrokeratoderma and alopecia. Other features include susceptibility to infections, dental dysplasia, hyperhidrosis and delayed growth; however, not all features have to be present to make the diagnosis. [Bettoli, 2021]

Systemic antibiotics and surgery for HS and DCS were reportedly most successful in contrast to isotretinoin, which was unsuccessful in four cases. In one case where treatment with isotretinoin, prednisolone and dicloxacillin failed; alitretinoin was ultimately used with good effect. [Maintz, 2005]

1.8 PAPASH

Co-occurrence of pyogenic sterile arthritis, pyoderma gangrenosum (PG) and acne was first defined as the PAPA syndrome in 1997. [Gottlieb, 2019]

It is a rare autosomal dominantly inherited disorder, caused by a mutation in the PSTPIP1 gene. [Kotzerke, 2021] Mutations (e.g. A230T and E250Q) may alter PSTPIP1-

encoded protein's interaction with phosphatases, resulting in hyperphosphorylation, which in turn promotes neutrophil-mediated inflammation, interleukin (IL)-1β and IL-17 overproduction, and an autoinflammatory disease state.

In the Eurofever Registry, on March 2015, 27 patients were classified as having the PAPA syndrome. [Toplak, 2011]

The association between PAPA and HS was defined as the PAPASH (pyogenic sterile arthritis, pyoderma gangrenosum, acne and suppurative hidradenitis) syndrome by Marzano et al. in 2013. [Marzano, 2013] PSTPIP1 mutations were found in two patients, who have been treated effectively with anakinra. [Kotzerke, 2021]

In the absence of pyogenic arthritis and PSTPIP1 mutations, the abbreviations PAPASH and PsAPASH have been used. The letters PA and PsA stand for *p*soriasis and *a*rthritis and *ps*oriatic *a*rthritis, respectively. As that differs from the previous definitions, they are included in the PASH section below.

1.9 PASS

The name PASS syndrome was proposed in 2012, [Bruzzese, 2012] when reporting a patient with co-occurring *p*yoderma gangrenosum, *a*cne conglobata, hidradenitis *s*uppurativa and axial *s*pondyloarthritis. A similar case was reported in 2015. [Leuenberger, 2012] No genetic testing was performed in the first case and no PSTPIP1 mutation found in the second, and no additional etiopathogenic clues are provided. The first patient responded to treatment with infliximab and isoniazid [Bruzzese, 2012] and the second to anakinra. [Leuenberger, 2012]

1.10 PASH

PASH syndrome was first defined in 2012 by Braun-Falco et al. [Braun-Falco, 2012] as a coexistence of pyoderma gangrenosum acne and *s*uppurative *h*idradenitis. PASH differs from the PAPA syndrome by the absence of pyogenic arthritis; however,

overlapping cases have been reported. [Genovese, 2020] An increased number of CCTG repeats in the PSTPIP1 gene promoter was found in five cases of PASH, still, it is considered a promoting rather than a causal factor. [Marzano, 2017] New evidence suggests that PASH is a polygenic entity, associated with cutaneous IL-1β, IL-17 and tumour necrosis factor-α (TNF-α) overexpression. [Marzano, 2017]

PASH is characterized by a male preponderance and a median age of 34 years. Three cases were associated with inflammatory bowel disease. [Marzano, 2014] PASH usually started in adolescence with the onset of acne conglobata, followed by HS and later PG. PG generally responded to treatment better than HS; however, the response was highly variable. Good response was reported after treatment with infliximab, adalimumab and anakinra. Two cases, [Jennings, 2017] resistant to TNF blockage, responded to anakinra. Join-Lambert et al. [Join-Lambert, 2015] treated four cases with prolonged targeted antibiotic therapy and surgery, achieving full control of both HS and PG lesions. Generally, the effects of antibiotics are not consistent. Ciclosporin and intralesional triamcinolone provided partial benefit for both HS and PG, while systemic steroids, dapsone, isotretinoin and topical tacrolimus produced mixed results.

1.11 SAPHO

The co-occurrence of *s*ynovitis, *a*cne, *p*ustulosis palmoplantaris, *h*yperostosis and *o*steitis was defined in 1987 as the SAPHO syndrome. [Chamot, 1987] It is considered a rare condition, affecting <1 per 10 000. Skin conditions such as acne conglobata, palmoplantar pustulosis and HS have been reported associated with the disease but are not compulsory for the diagnosis. [Fania, 2020]

In SAPHO, patients present with osteoarticular involvement, soft tissue swelling and limited range of motion. The anterior chest wall and spine are the most commonly affected, but appendicular and mandibular sites are reported as well, along with synovitis. The skin manifestations are neutrophilic dermatoses, most commonly

palmoplantar pustulosis, acne conglobata, psoriasis and HS. [Nguyen, 2012] Similarly to HS, SAPHO may be associated with inflammatory bowel disease. [Naves, 2013]

Etiopathogenesis of SAPHO is not clear. IL-1β, IL-8, IL-17 and TNF-α are believed to play a major role. Furthermore, the increased release of IL-1β is hypothesized to be due to the P2X7 receptor dysregulation. SAPHO is associated to T309G allele and the GG-genotype of the Mdm2-gene, resulting in altered p53 response. [Przepiera-Będzak, 2021]

Infliximab and other TNF-α inhibitors appear to resolve the osteoarticular symptoms of SAPHO more effectively than the associated HS, but a reduction in SAPHO activity was followed by a reduction in HS symptoms. The link between the activity of HS and SAPHO supports the existence of a causal relationship and treatment of either appears to alleviate both. [Genovese, 2019]

1.12 Syndromic HS and familial Mediterranean fever

Occurrence of HS has been reported in patients affected by FMF, one of the prototypical and most common monogenic autosomal recessive AIDs, characterized by recurrent attacks of fever, peritonitis, arthritis, and amyloidosis. [Hodak, 2017] Epidemiologic evidence from a case-control study has documented an increased prevalence of FMF (0.7% vs 0.1% in controls) in a cohort of 4417 patients with HS, suggesting the association between AIDs and HS. [Hodak, 2017] In some patients with the HS-FMF overlap, disease activity of HS paralleled the attacks of FMF, whereas in others HS presented a persistent course irrespective of FMF activity. [Vural, 2017]

Patients with HS-FMF carried also heterozygous mutations in the *MEFV* gene. A recent case-control study confirmed this finding, reporting an increased prevalence of pathogenic *MEFV* variants in patients with a complex HS phenotype. This was defined as isolated Hurley III disease or Hurley II disease in association with other inflammatory conditions (pyoderma gangrenosum, arthritis, inflammatory bowel disease,

or acne conglobata). [Vural, 2019] Such clinical and genetic association reinforces the importance of an autoinflammatory component in the pathogenesis of moderate-severe HS, such as may be observed in rheumatic, inflammatory bowel, and neutrophilic disease.

2 Materials and methods

2.1 Patients

From January 2011 to May 2019, 10 patients with syndromic HS were enrolled. All patients signed a written informed consent after the approval by the Area B Milan Ethics Committee (protocol no. 487_2020).

2.2 Genomic DNA extraction, Whole Exome Sequencing and Data analysis

The genomic DNAs were extracted from saliva samples using Oragene-DNA (Ottawa, Canada) kit following the manufacturer's instructions. Agarose gel and Qubit (Invitrogen, Oregon USA) evaluated the quantity and quality of the extraction. Whole Exome Sequencing (WES) library preparation and sequencing, with averaged 100x of expected coverage has been performed in outsourcing service by Macrogen (Seoul, Korea) with Illumina® HiSeq 2500 System.

WES was performed through a pipeline already described by Brandao *et al*.[18] Following, the sequencing coverage was re-calculated and resulted in an average of 93.9%, 36.0% and 9.3% for 10, 50, 100x coverage, respectively. Adapter trimming, short and low-quality reads was filtered out using the Trim Galore v0.39, using default parameters (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/).[20] The read alignment was carried out using Burrows-Wheeler Aligner (BWA) Software Package, using the Human Genome version 38 (GRCh.38) as reference genome. Duplicate removal and base recalibration were made by Picard tools (https://broadinstitute.github.io/picard/)[21] and GATK v. 4.1.2.0 (https://software.broadinstitute.org/gatk/),[22] respectively. Variant calling and filtration were also made using GATK v. 4.1.2.0. ANNOVAR software for gene-based and filter-based variant annotations (dbSNP 151, CADD; GERP++, SIFT, PolyPhen2, FATHMM, COSMIC70, ClinVar, 1000 Genomes Project, ExAC 03, genomicsSuperDups, wgRNA, GWAS Catalog, and Interpro) relative to the GRCh.38 genome was used. An in-house

pipeline was made, using R Software, to analyze the annotated exome data. The investigation concentrated on variants in coding regions (ExonVar), including those related to exon splicing. We retrieved the distribution, density, and description of ExonVar, including single nucleotide polymorphism (SNP) and insertion/deletion (INDEL) for each patient. An impact mutational score was developed to determine the variant with potential impact on the protein by assembling multiple algorithmic prediction results, including SIFT, PolyPhen2, FATHMM. A value 1 was imputed in the impact mutational score each time when the algorithm predicts a damage impact. Then we add the CADD values 15 in the score. Variants carrying an assembly >= 5 or CADD > 15 were considered a potential impact variation (ImpactVar). Two approaches were employed to better understand PASH etiopathogenesis. First, variations in genes of the gamma-secretase complex were searched (*NCSTN*, *PSENEN* and *PSEN1*); secondly, homozygosis rare ExonVar, were analyzed.

3 Results

3.1 Clinical features and treatment

Ten syndromic HS patients [5 men, 5 women] were studied. Seven patients, three of whom had a form overlapping with SAPHO, were affected by PASH and three by PAPASH. Clinical features of syndromic HS patients are summarized in **Table I**. Family history for HS or PG was negative for all patients. Based on the presence of gut, joint and gut/joint inflammation, the following three syndromic HS settings were identified: gut inflammation (n=4; Crohn's disease [n=2], bowel bypass syndrome [n=2]), joint inflammation (n=5; SAPHO syndrome [n=3]; pyogenic arthritis [n=2]) and gut/joint inflammation (n=1; ulcerative colitis and pyogenic arthritis) (**Table I**).

Table I. Clinical features of patients with syndromic hidradenitis suppurativa

	ID	Sex	Diagnosis	Comorbidities	Hidradenitis suppurativa			Pyoderma gangrenosum			Acne			Systemic treatments/surgery	Follow-up
					Age at onset (years)	Involved sites	IHS4	Age at onset (years)	Involved sites	PG Score	Age at onset (years)	Involved sites	GAGS score		
JOINT INFLAMMATION	1	F	PAPASH	FMF, PCOS, pyogenic arthritis	16	axillae, groins, anogenital area, intermammary and submammary folds	20	16	trunk	multilesional	14	face	25	azitromycine, doxycycline, dapsone, adalimumab, anakinra, colchicine	complete response
	2	M	PAPASH	pyogenic arthritis	14	axillae, groins, back, nuchal region, scalp	30	18	trunk	multilesional	13	face, back	32	rifampicin, doxycycline, azitromycine, lymecycline, clindamycin, adalimumab, anakinra, prednisone	complete response
	3	M	PASH/ SAPHO	SAPHO	43	axillae, groins, anogenital area	27	43	lower limbs, trunk, upper limbs	disseminated	17	face, back	39	clindamycin, infliximab	complete response
	4	M	PASH/ SAPHO	SAPHO	42	axillae, groins, anogenital area	19	46	lower limbs	localized	16	face	24	clindamycin, methotrexate, infliximab, adalimumab	complete response
	5	F	PASH/ SAPHO	SAPHO	15	axillae, groins, anogenital region, nuchal region	26	15	trunk	multilesional	13	face	28	rifampicin, doxycycline, azitromycine, lymecycline, clindamycin, intravenous immunoglobulin, methotrexate, isotretinoin, adalimumab, anakinra, ustekinumab, secukinumab	no response
GUT/J	6	F	PAPASH	Ulcerative colitis, PCOS, pyogenic arthritis	28	submammary folds, groins, anogenital area	22	36	peristomal	localized	15	face, back	23	doxycycline, infliximab, surgery for HS	complete response

OINT															
GUTINFLAMMATION	7	M	PASH	Obesity, BBS, osteoporosis, PPPP	36	axillae, groins, anogenital area	24	36	lower limbs, trunk, upper limbs	disseminated	34	face	30	trimetoprim-sulfametoxazole, azitromycine, cyclosporine, prednisone, adalimumab, ustekinumab,	complete response
	8	M	PASH	Crohn disease, osteoporosis	18	axillae, groins, anogenital area, nuchal region	23	19	lower limbs	multilesional	16	face, back	31	azitromycine, trimetoprim-sulfametoxazole, ceftriaxone, ciprofloxacine, lymecycline, rifampicin, amoxicillin clavulanate, methylprednisolone, dapsone, cyclosporine, adalimumab, anakinra,	partial response
	9	F	PASH	Crohn disease, coeliac disease, myasthenia gravis, PCOS	18	axillae, nuchal region, submammary folds, groins, anogenital area	19	44	peristomal	localized	17	face, back	26	clindamycin, methotrexate, infliximab, adalimumab, surgery for HS	complete response
	10	F	PASH	Obesity, BBS, myocardial infarction, type II DM, BP	51	axillae, groins, submammary folds, anogenital area	23	51	perianal region	localized	15	face	27	rifampicin, doxycycline, infliximab, adalimumab	complete response

Abbreviations: BBS, bowel bypass syndrome; BP, bullous pemphigoid; DM, diabetes mellitus; FMF, familial Mediterranean fever; PAPASH, pyogenic arthritism pyoderma gangrenosum, acne and hidradenitis suppurativa; PASH, pyoderma gangrenosum, acne and hidradenitis suppurativa; PCOS, polycystic ovarian syndrome; PPPP, palmoplantar pustular psoriasis; SAPHO, synovitis, acne, hyperostosis and osteitis

Mean age at HS onset was 28.1 years. The disease was chronic-relapsing in all cases. The most frequently involved sites were the axillae (n=9), groins (n=9) and anogenital region (n=9), followed by submammary/intermammary folds (n=4), nuchal region (n=3) and scalp (n=1). Mean IHS4 was 23.3. PG was ulcerative in all cases, being associated with vegetating aspects in four cases. Age at PG onset generally paralleled age at HS onset (mean age at PG onset: 32.4 years), with a mean difference between them of 4.1 years. The most frequently involved site was represented by the trunk (n=5), followed by lower limbs (n=4), upper limbs (n=2), peristomal site (n=2) and perianal region (n=1). It was localized in four cases, multilesional in four and disseminates in two. Acne onset predated the appearance of HS/PG lesions in all patients (mean age at onset: 17 years), with a mean time between acne and PG/HS onset of 11 years and a mean GAGS of 28.5. One of the patients (P7) with disseminated PG had also widespread HS. This patient, together with another one (P2), had also very severe HS involving typical and atypical sites. Widespread acne-like HS and severe acne were present also in a patient (P5) with very refractory cutaneous manifestations leading to multiple lines of biological and immunosuppressive treatments without complete control of the overall HS picture. Arthritis of the wrists and ankles was present in the three patients with PAPASH, while the three patients with PASH/SAPHO overlapping showed sacroiliac joint, ankle and spine involvement. Mean age of arthritis onset was 27.5 years. All patients were treated with topical therapy, mainly represented by clindamycin gel for HS lesions and high-potency corticosteroids for PG lesions. Systemic antibiotics were given to all patients, while oral retinoids (isotretinoin) were administered only to one patient. Immunosuppressive/immunomodulating agents were given to five patients. Biologic agents were administered to all patients. Surgery for HS was performed in two patients.

3.2 Genetic findings

3.2.1 Exome Description: Overall

In overall analysis, 343168 different variations were found in our HS patients of whom 57707 (16.81%) were ExonVar. **Table II** shows the list and type of variations identified for each patient. Mean number of global and ExonVar was 122960 and 57825, respectively. Most ExonVar were localized on chromosome 1, 2 and 19. ExonVar were distributed on 14546 genes, the most frequent (27966) being nonsynonymous (ns) ExonVar with a possible detrimental action on 10356 genes (**Table III**).

Table II. Distribution of the individual variants according to their location.

Location	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
downstream	929	854	944	2867	2465	2236	2137	3243	2895	2311
exonic	23616	23262	22904	23890	24053	23679	22847	24015	23648	23843
exonic;splicing	15	11	8	13	10	16	14	13	13	19
intergenic	215115	237577	256111	112833	82604	74493	73367	138631	122702	84509
intronic	77441	63256	67870	153678	135736	122904	122751	168771	158148	132237
ncRNA_exonic	2816	2733	2814	4021	3727	3677	3575	4182	4142	3861
ncRNA_exonic;splicing	4	5	2	2	1	1	5	3	5	4
ncRNA_intronic	4984	4605	4875	14719	11858	10497	10473	16226	15318	11544
ncRNA_splicing	10	12	14	21	15	17	21	17	20	28
splicing	85	67	76	76	95	95	78	95	87	92
upstream	2392	2041	2267	4922	4494	4239	4030	5270	5189	4630
upstream;downstream	169	126	155	317	270	262	225	313	310	264
UTR3	4296	3594	3780	6935	6482	5955	5879	7165	6980	6420
UTR5	2628	2328	2492	3322	3213	2987	2939	3313	3359	3296
UTR5; UTR3	4	4	8	9	8	9	9	5	11	7

Table III. Exonic variants (ExonVar) per sample according to their type

Type of variations	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
frameshift deletion	141	144	132	154	144	148	143	154	138	145
frameshift insertion	102	109	97	101	110	96	107	106	107	109
nonframeshift deletion	207	203	198	213	206	192	202	212	205	217
nonframeshift insertion	174	151	158	173	179	175	162	174	175	172
nonsynonymous SNV	10862	10787	10569	11083	10962	10884	10529	11048	10977	11109
stopgain	104	102	81	104	103	93	97	93	96	96
stoploss	15	14	9	12	15	16	14	11	14	10
synonymous SNV	11798	11545	11388	11806	12107	11845	11339	11959	11719	11739
.	140712	126509	133647	327284	274742	250779	248097	370928	342488	272771
unknown	213	207	272	232	219	226	245	248	208	235

3.2.2 ExonVar approach

We filtered ExonVar by Minor Allele Frequency (MAF) and impactVar. A total of 10268 ExonVar (representing 17.76% of the kind of variant) were rare (GnomAD v3.0 MAF <= 0.01) and 28553 (49.38%) with potential mutational impact. Only 3188 (5.51%) ExonVar were rare and with potential mutational impact. Then, we focused on homozygous mutations (**Table IV**): forty-nine rare mutations with functional impact were found in 43 *loci,* comprising 45 genes.

Table IV. Count of homozygotic mutations in each patient.

Patient	**ExonVar_homozygotic_rare**	**ExonVar_homozygotic_rare_mutational impact**
P1	49	1
P2	57	4
P3	85	10
P4	46	3
P5	39	1
P6	62	6
P7	128	20
P8	74	3
P9	40	0
P10	53	7

3.2.3 Genetic variants in autoinflammation and keratinization genes

Genetic variants were discovered in seven genes implicated in autoinflammation, including *MEFV*, *PSTPIP1*, *NLRC4*, *WDR1*, NO*D2*, *MPO* and *OTULIN*, and two genes regulating the keratinization process, including *NCSTN* and *GJB2*. (**Table V**) Among syndromic HS patients with joint inflammation, three were affected by PAPASH and three by PASH/SAPHO overlapping. A patient with PAPASH (P1) was also affected by FMF and exhibited a missense mutation in *PSTPIP1* gene (p.E277D) as well as two pathogenic variants in *MEFV* gene (p.M694V and p.V726A) linked to the diagnosis of FMF. Two PAPASH patients (P2 and P6) had a novel frameshift *NCSTN* variant (p.D381Sfs*7) and a variant of uncertain significance (VUS) in *NLRC4* gene (p.R181X), respectively. Eventually, a previously unreported variant in *WDR1* gene (p.H108R) in a patient (P5) with PASH/SAPHO overlapping with widespread HS and severe acne conglobata, which were extremely treatment-refractory. The patient P2 with a novel variant in *NCSTN* gene had an extensive cutaneous involvement with ulcerative and vegetating PG and severe HS. Among syndromic HS patients with gut inflammation, three were affected by IBD, one of whom has also coeliac disease, and two underwent bowel bypass surgery for morbid obesity. Three PASH patients (P7, P8, and P10) showed three different variants in *NOD2* gene (p.R702W, p.L1007Pfs*2 and p.G908R). Genetic variants in the *OTULIN* gene (p.I70T) and (p.Q115H) were found in two patients with gut inflammation (P7 and P9). In patients with gut inflammation, we observed also genetic changes in the *MPO* gene (p.M251T) in patient 7 and in the *GJB2* gene (p.G12Vfs*2) in patient 9. The only patient with joint and gut inflammation (P6) had a VUS in *NLRC4* gene (p.R181X).

Table V. Pathogenic variants, VUS and variants not yet reported in autoinflammatory genes

	ID	Sex	Diagnosis	Gene	Change in DNA	Change in protein	Clinical significance
JOINT INFLAMMATION	1	F	PAPASH	MEFV	c.2080A>G	p.(M694V)	Pathogenic
				MEFV	c.2177T>C	p.(V726A)	Pathogenic

				PSTPIP1	c.831G>T	p.(E277D)	Pathogenic
	2	M	PAPASH	NCSTN	c.1140_1141del	p.(D381Sfs*7)	Not yet reported
	3	M	PASH/SAPHO	NCSTN	c.482delA	p.(I162Yfs*57)	Not yet reported
	4	M	PASH/SAPHO	NLRC4	c.2668T>C	p.(C890R)	Variant of Uncertain Significance
	5	F	PASH/SAPHO	WDR1	c.323A>G	p.(H108R)	Not yet reported
GUT/JOINT	6	F	PAPASH	NLRC4	c.541C>T	p.(R181X)	Variant of Uncertain Significance
GUT INFLAMMATION	7	M	PASH	NOD2	c.2104C>T	p.(R702W)	Risk factor for IBD
				MPO	c.752T>C	p.(M251T)	Conflicting interpretation of pathogenicity (Pathogenic AND Variant of Uncertain Significance)
				OTULIN	c.209T>C	p.(I70T)	Variant of Uncertain Significance
	8	M	PASH	NOD2	c.3017dupC	p.(L1007Pfs*2)	Risk factor for IBD
	9	F	PASH	GJB2	c.35delG	p.(G12Vfs*2)	Pathogenic
				OTULIN	c.345G>T	(p.Q115H)	Variant of Uncertain Significance
	10	F	PASH	NOD2	c.2722G>C	p.(G908R)	Susceptibility factor to IBD

Abbreviations: IBD, inflammatory bowel disease PAPASH, pyogenic arthritism pyoderma gangrenosum, acne and hidradenitis suppurativa; PASH, pyoderma gangrenosum, acne and hidradenitis suppurativa; SAPHO, synovitis, acne, hyperostosis and osteitis

4 Discussion

We report here 10 patients with syndromic HS. All the patients had severe HS (mean IHS4 > 11 and Hurley III) and localized, multilesional or disseminated PG All the patients underwent treatment with different biologics experiencing several flares of HS/PG.

Three phenotypes were identified based on the predominance of gut inflammation (IBD/bowel bypass syndrome), joint inflammation (arthritis) or coexistence of gut and joint inflammation.

The four PASH patients with gut inflammation showed three different variants in *NOD2* gene, a variant in *GJB2* gene, a variant in *MPO* gene and two variants in *OTULIN* gene.

NOD2 gene variants, classically associated with Crohn disease, [McGovern, 2001] have been reported to be related to NOD2-associated autoinflammatory disease,

characterized by infiltrated skin lesions and gastrointestinal symptoms with recurrent fever and arthralgia. [Yao, 2019]

Several studies demonstrated an association of IBD with HS and PG, supporting the hypothesis that similar pathogenic mechanisms contribute to these diseases and paving the way to a common targeted therapy. [van der Zee, 2010; Shalom, 2016; Yada, 2016]

IBD, PG and HS are all associated with disrupted immune tolerance related to gut dysbiosis and the link between gut inflammation, innate immunity dysregulation and autoinflammatory skin disorders is further supported by the reports of HS development in patients with bowel bypass surgery-associated altered gut microbiota. [Marzano, 2012; Garcovich, 2019] Otulin gene encodes a ubiquitin isopeptidase participating to NFkB-dependent inflammatory signalling. Loss-of-function mutations in Otulin were associated in a mouse model with OTULIN-related autoinflammatory syndrome, a condition characterized by skin nodules and gastrointestinal inflammation with fever and arthritis. [Damgaard, 2016] Our finding of two variants in Otulin may support a contribution of this gene in skin and gut inflammation.

MPO gene encodes myeloperoxidase, crucial for neutrophil antimicrobial activity. Its deficiency results in an exaggerated inflammatory response and is associated with pustular psoriasis, [Aratani, 2018] as seen in our patient (P7) showing this variation. This patient had also a bowel bypass syndrome, whose association with PASH can be explained by the disturbance of gut microbiome. [Marzano, 2012; Garcovich, 2019]

Several hypotheses can be drawn when considering the pathomechanisms underlying this clinical association: (i) the role of micronutrient deficiencies (zinc, iron, vitamin D, vitamin A) as regulators of innate immune responses and follicular hyperkeratosis; (ii) the disturbance of the gut microbiome; (iii) adipokine dysregulation; (iv) alteration of the perifollicular, dermal extracellular matrix (ECM) and/or augmented skin friction due to excess skin and contour deformities.

Each of these factors can be induced by BS intervention and substantial weight loss, especially in the case of malabsorptive procedures, thus potentially contributing to the dysregulation of follicular, innate immune responses.

GJB2 encodes the gap junction protein connexin 26 (Cx26) that regulates either the epidermis and hair follicle keratinization. *GJB2* mutations cause Keratitis-ichthyosis-deafness syndrome, an inherited ectodermal disorder reported in association with the triad HS, acne conglobata and scalp dissecting folliculitis. We found a variant in *GJB2* suggesting that an altered keratinization pathway may play a role in syndromic HS pathogenesis. [Maintz, 2005]

Three PAPASH and three PASH/SAPHO overlapping patients with joint inflammation showed two different novel frameshift variants in *NCSTN* gene, a variant in *WDR1* and in *PSTPIP1* gene and two variants in *NLRC4* gene, one of whom was present in a patient with a mixed phenotype characterized by gut and joint inflammation. A patient with PAPASH and FMF showed pathogenic variants in *MEFV* gene responsible for FMF.

Mutations of genes encoding for the γ-secretase protein complex have been reported in familial HS. [Vossen, 2020] Interestingly, we found two novel variants in NCSTN gene in one non-familial PAPASH patient and one with PASH/SAPHO overlapping Pathogenic variants of this gene have been previously reported in non-familial PASH [Duchatelet, 2015] and in SAPHO presenting with HS manifestations, [Li, 2018] indicating that not only familial but also syndromic HS pathogenesis may be linked to NCSTN variants.

WDR1 mutations is cause autoinflammatory disease and neutrophilia. Intriguingly, we found a novel variant in a PASH/SAPHO overlapping patient with severe and refractory HS. [Kile, 2007]

Gain of function mutations in *NLCR4* gene have been related to inflammasomopathies. [Canna, 2014] In our series, NLRC4 variants were found in patient with joint inflammation and in another one with both gut and joint inflammation.

The mutation (E277D) of PSTPIP1 gene, whose discovered mutations are typically involved in PAPA syndrome, [Wise, 2002] has been previously reported by us in a PAPASH patient [Marzano, 2017] and has been confirmed in the same patient in the present study. In the light of the association between this gene and PAPA, we hypothesized that *PSTPIP1* variations may be linked to joint autoinflammation. The same patient was affected by FMF, making it not surprising discovering two pathogenic variants in *MEFV*. Given that the frequency of MEFV mutations in patients with HS is higher than that in healthy controls, [Vural, 2017] it is likely that mutations of this gene may contribute also to the pathogenesis of HS and its syndromic forms.

These findings collectively corroborate the polygenic autoinflammatory nature of syndromic HS that is closely linked to joint and gut inflammation. A genetically disrupted keratinization pathway may contribute to the pathogenesis of skin inflammation in syndromic HS. WES should be recommended as a key tool for correctly diagnosing and classifying these conditions. Future research on the molecular basis of syndromic HS with functional validation of discovered mutations could pave the way to pathogenesis-targeted treatment.

5 References

1. Jemec GB. Clinical practice. Hidradenitis suppurativa. N Engl J Med 2012; 366: 158– 64.

2. Saunte DM, Boer J, Stratigos A et al. Diagnostic delay in hidradenitis suppurativa is a global problem. Br J Dermatol 2015; 173: 1546– 9.

3. Kolli SS, Senthilnathan A, Cardwell LA et al. Hidradenitis suppurativa has an enormous impact on patients' lives. J Am Acad Dermatol 2020; 82: 236– 8.

4. Lima AL, Karl I, Giner T et al. Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. Br J Dermatol 2016; 174: 514– 21.

5. Marzano AV, Damiani G, Ceccherini I et al. Autoinflammation in pyoderma gangrenosum and its syndromic form (pyoderma gangrenosum, acne and suppurative hidradenitis). Br J Dermatol 2017; 176: 1588– 98.

6. Micheletti RG. Hidradenitis suppurativa: current views on epidemiology, pathogenesis, and pathophysiology. Semin Cutan Med Surg. 2014 Jun;33(3 Suppl):S48-50. doi: 10.12788/j.sder.0091.

7. Theut Riis P, Loft IC, Yazdanyar S, Kjaersgaard Andersen R, Pedersen OB, Ring HC, Huber R, Sultan M, Loesche C, Saunte DML, Jemec GBE. Full exome sequencing of 11 families with Hidradenitis suppurativa. J Eur Acad Dermatol Venereol. 2021 May;35(5):1203-1211. doi: 10.1111/jdv.17095.

8. Wang Z, Yan Y, Wang B. γ-Secretase Genetics of Hidradenitis Suppurativa: A Systematic Literature Review. Dermatology. 2021;237(5):698-704. doi: 10.1159/000512455.

9. Gasparic J, Theut Riis P, Jemec GB. Recognizing syndromic hidradenitis suppurativa: a review of the literature. J Eur Acad Dermatol Venereol. 2017 Nov;31(11):1809-1816.

10. Higgins R, Pink A, Hunger R, Yawalkar N, Navarini AA. Generalized Comedones, Acne, and Hidradenitis Suppurativa in a Patient with an FGFR2 Missense Mutation. Front Med (Lausanne). 2017 Feb 28;4:16. doi: 10.3389/fmed.2017.00016.

11. Vasanth V, Chandrashekar BS. Follicular occlusion tetrad. Indian Dermatol Online J. 2014 Oct;5(4):491-3. doi: 10.4103/2229-5178.142517.

12. AlSabbagh MM, Baqi MA. Bazex-Dupré-Christol syndrome: review of clinical and molecular aspects. Int J Dermatol. 2018 Sep;57(9):1102-1106. doi: 10.1111/ijd.14065.

13. Stephan C, Kurban M, Abbas O. Dowling-Degos disease: a review. Int J Dermatol. 2021 Aug;60(8):944-950.

14. Lam M, Lai C, Almuhanna N, Alhusayen R. Hidradenitis suppurativa and Down syndrome: A systematic review and meta-analysis. Pediatr Dermatol. 2020 Nov;37(6):1044-1050. doi: 10.1111/pde.14326.

15. Blok J, Jonkman M, Horváth B. The possible association of hidradenitis suppurativa and Down syndrome: is increased amyloid precursor protein expression resulting in impaired Notch signalling the missing link? Br J Dermatol. 2014 Jun;170(6):1375-7. doi: 10.1111/bjd.12887.

16. Bettoli V, Forconi R, Pezzini I, Martinello R, Scuderi V, Zedde P, Schettini N, Pacetti L, Corazza M. KID Syndrome and Hidradenitis Suppurativa: A Rare Association Responding to Surgical Treatment. Skin Appendage Disord. 2021 Jan;7(1):21-24. doi: 10.1159/000509042.

17. Maintz L, Betz RC, Allam JP, Wenzel J, Jaksche A, Friedrichs N, Bieber T, Novak N. Keratitis-ichthyosis-deafness syndrome in association with follicular occlusion triad. Eur J Dermatol. 2005 Sep-Oct;15(5):347-52.

18. Gottlieb J, Madrange M, Gardair C, Sbidian E, Frazier A, Wolkenstein P, Hickman G, Schneider P, Baudry C, Claudepierre P, Bertheau P, Richette P, Smahi A, Bachelez H. PAPASH, PsAPASH and PASS autoinflammatory syndromes: phenotypic heterogeneity, common biological signature and response to immunosuppressive regimens. Br J Dermatol. 2019 Oct;181(4):866-869.

19. Kotzerke M, Mitri F, Marbach F, Enk A, Haenssle H. A case of PAPASH syndrome in a young man carrying a novel heterozygote missense variant in PSTPIP1. J Eur Acad Dermatol Venereol. 2021 Jul;35(7):e439-e440. doi: 10.1111/jdv.17171.

20. Toplak N, Frenkel J, Ozen S, De Benedetti F, Hofer M, Kone-Paut I, Girschick H, Neven B, Ozdogan H, Kummerle-Deschner J, Arostegui J, Simon A, Stojanov S, Vesely R, Wouters C, Hentgen V, Rose C, Dolezalova P, Lachmann H, Woo P, Touitou I, Martini A, Ruperto N, Gattorno M. The Eurofever Registry for autoinflammatory diseases: results of the first 15 months of enrolment. Pediatr Rheumatol Online J. 2011 Sep 14;9(Suppl 1):P303. doi: 10.1186/1546-0096-9-S1-P303.

21. Marzano AV, Trevisan V, Gattorno M, Ceccherini I, De Simone C, Crosti C. Pyogenic arthritis, pyoderma gangrenosum, acne, and hidradenitis suppurativa (PAPASH): a new autoinflammatory syndrome associated with a novel mutation of the PSTPIP1 gene. JAMA Dermatol. 2013 Jun;149(6):762-4. doi: 10.1001/jamadermatol.2013.2907.

22. Bruzzese V. Pyoderma gangrenosum, acne conglobata, suppurative hidradenitis, and axial spondyloarthritis: efficacy of anti-tumor necrosis factor α therapy. J Clin Rheumatol. 2012 Dec;18(8):413-5. doi: 10.1097/RHU.0b013e318278b84c.

23. Leuenberger M, Berner J, Di Lucca J, Fischer L, Kaparos N, Conrad C, Hohl D, So A, Gilliet M. PASS Syndrome: An IL-1-Driven Autoinflammatory Disease. Dermatology. 2016;232(2):254-8. doi: 10.1159/000443648.

24. Braun-Falco M, Kovnerystyy O, Lohse P, Ruzicka T. Pyoderma gangrenosum, acne, and suppurative hidradenitis (PASH)--a new autoinflammatory syndrome distinct from PAPA syndrome. J Am Acad Dermatol. 2012 Mar;66(3):409-15. doi: 10.1016/j.jaad.2010.12.025.

25. Jennings L, Molloy O, Quinlan C, Kelly G, O'Kane M. Treatment of pyoderma gangrenosum, acne, suppurative hidradenitis (PASH) with weight-based anakinra dosing in a Hepatitis B carrier. Int J Dermatol. 2017 Jun;56(6):e128-e129. doi: 10.1111/ijd.13528.

26. Genovese G, Moltrasio C, Garcovich S, Marzano AV. PAPA spectrum disorders. G Ital Dermatol Venereol. 2020 Oct;155(5):542-550. doi: 10.23736/S0392-0488.20.06629-8.

27. Marzano AV, Ceccherini I, Gattorno M, Fanoni D, Caroli F, Rusmini M, Grossi A, De Simone C, Borghi OM, Meroni PL, Crosti C, Cugno M. Association of pyoderma gangrenosum, acne, and suppurative hidradenitis (PASH) shares genetic and cytokine profiles with other autoinflammatory diseases. Medicine (Baltimore). 2014 Dec;93(27):e187. doi: 10.1097/MD.0000000000000187.

28. Join-Lambert O, Duchatelet S, Delage M, Miskinyte S, Coignard H, Lemarchand N, Alemy-Carreau M, Lortholary O, Nassif X, Hovnanian A, Nassif A. Remission of refractory pyoderma gangrenosum, severe acne, and hidradenitis suppurativa (PASH) syndrome using targeted antibiotic therapy in 4 patients. J Am Acad Dermatol. 2015 Nov;73(5 Suppl 1):S66-9. doi: 10.1016/j.jaad.2015.07.040.

29. Przepiera-Będzak H, Brzosko M. SAPHO syndrome: pathogenesis, clinical presentation, imaging, comorbidities and treatment: a review. Postepy Dermatol Alergol. 2021 Dec;38(6):937-942. doi: 10.5114/ada.2020.97394.

30. Chamot AM, Benhamou CL, Kahn MF, Beraneck L, Kaplan G, Prost A. Le syndrome acné pustulose hyperostose ostéite (SAPHO). Résultats d'une enquête nationale. 85 observations [Acne-pustulosis-hyperostosis-osteitis syndrome. Results of a national survey. 85 cases]. Rev Rhum Mal Osteoartic. 1987 Mar;54(3):187-96.

31. Fania L, Moro F, Clemente A, Sordi D, Scarabello A, Mazzanti C, Caggiati A, Zecchi V, Panebianco A, Cassotta G, Abeni D. Successful treatment of Sapho syndrome and hidradenitis suppurativa: A therapeutic challenge. Dermatol Ther. 2020 May;33(3):e13453. doi: 10.1111/dth.13453.

32. Naves JE, Cabré E, Mañosa M, Grados D, Olivé A, Domènech E. A systematic review of SAPHO syndrome and inflammatory bowel disease association. Dig Dis Sci. 2013 Aug;58(8):2138-47. doi: 10.1007/s10620-013-2653-6.

33. Nguyen MT, Borchers A, Selmi C, Naguwa SM, Cheema G, Gershwin ME. The SAPHO syndrome. Semin Arthritis Rheum. 2012 Dec;42(3):254-65. doi: 10.1016/j.semarthrit.2012.05.006.

34. Genovese G, Caorsi R, Moltrasio C, Marzano AV. Successful treatment of co-existent SAPHO syndrome and hidradenitis suppurativa with adalimumab and methotrexate. J Eur Acad Dermatol Venereol. 2019 Oct;33 Suppl 6:40-41. doi: 10.1111/jdv.15849.

35. Hodak E, Atzmony L, Pavlovsky L, Comaneshter D, Cohen AD. Hidradenitis Suppurativa Is Associated with Familial Mediterranean Fever-A Population-Based Study. J Invest Dermatol. 2017 Sep;137(9):2019-2021. doi: 10.1016/j.jid.2017.04.024.

36. Vural S, Gundogdu M, Kundakci N, Ruzicka T. Familial Mediterranean fever patients with hidradenitis suppurativa. Int J Dermatol. 2017 Jun;56(6):660-663. doi: 10.1111/ijd.13503.

37. Vural S, Gündoğdu M, Gökpınar İli E, Durmaz CD, Vural A, Steinmüller-Magin L, Kleinhempel A, Holdt LM, Ruzicka T, Giehl KA, Ruhi HI, Boyvat A. Association of pyrin mutations and autoinflammation with complex phenotype hidradenitis suppurativa: a case-control study. Br J Dermatol. 2019 Jun;180(6):1459-1467. doi: 10.1111/bjd.17466.

38. McGovern DP, van Heel DA, Ahmad T, et al. NOD2 (CARD15), the first susceptibility gene for Crohn's disease. Gut. 2001;49: 752-4. doi: 10.1136/gut.49.6.752.

39. Yao Q, Li E, Shen B. Autoinflammatory disease with focus on NOD2-associated disease in the era of genomic medicine. Autoimmunity. 2019; 52:48-56. doi: 10.1080/08916934.2019.1613382.

40. van der Zee HH, van der Woude CJ, Florencia EF, et al. Hidradenitis suppurativa and inflammatory bowel disease: are they associated? Results of a pilot study. Br J Dermatol. 2010; 162:195-7. doi: 10.1111/j.1365-2133.2009.09430.x.

41. Shalom G, Freud T, Ben Yakov G, et al. Hidradenitis Suppurativa and Inflammatory Bowel Disease: A Cross-Sectional Study of 3,207 Patients. J Invest Dermatol. 2016; 136:1716-1718. doi: 10.1016/j.jid.2016.04.003.

42. Yada S, Singh S, Edakkanambeth Varayil, et al. Hidradenitis Suppurativa in Patients With Inflammatory Bowel Disease: A Population-Based Cohort Study in Olmsted County, Minnesota. Clin Gastroenterol Hepatol. 2016; 14: 65-70. https://doi.org/10.1016/j.cgh.2015.04.173.

43. Marzano AV, Ishak RS, Colombo A, et al. Pyoderma gangrenosum, acne and suppurative hidradenitis syndrome following bowel bypass surgery. Dermatology. 2012; 225:215-9. doi: 10.1159/000343602.

44. Garcovich S, De Simone C, Giovanardi G, et al. Post-bariatric surgery hidradenitis suppurativa: a new patient subset associated with malabsorption and micronutritional deficiencies. Clin Exp Dermatol. 2019; 44:283-289. doi: 10.1111/ced.13732.

45. Damgaard RB, Walker JA, Marco-Casanova P, et al. The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity. Cell. 2016;166 :1215-1230.e20. doi: 10.1016/j.cell.2016.07.019.

46. Aratani Y. Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys. 2018; 640:47-52. doi: 10.1016/j.abb.2018.01.004.

47. Maintz L, Betz RC, Allam JP, et al. Keratitis-ichthyosis-deafness syndrome in association with follicular occlusion triad. Eur J Dermatol. 2005; 15:347-52.

48. Vossen ARJV, van Straalen KR, Swagemakers SMA et al. A novel nicastrin mutation in a three-generation Dutch family with hidradenitis suppurativa: a search for functional significance. J Eur Acad Dermatol Venereol. 2020; 34:2353-2361. doi: 10.1111/jdv.16310.

49. Duchatelet S, Miskinyte S, Join-Lambert O, et al. First nicastrin mutation in PASH (pyoderma gangrenosum, acne and suppurative hidradenitis) syndrome. Br J Dermatol. 2015; 173:610-2. doi: 10.1111/bjd.13668.

50. Kile BT, Panopoulos AD, Stirzaker RA, et al. Mutations in the cofilin partner Aip1/Wdr1 cause autoinflammatory disease and macrothrombocytopenia. Blood. 2007; 110:2371-80. doi: 10.1182/blood-2006-10-055087.

51. Li C, Xu H, Wang B. Is SAPHO Syndrome Linked to PASH Syndrome and Hidradenitis Suppurativa by Nicastrin Mutation? A Case Report. J Rheumatol. 2018;45: 1605-1607. doi: 10.3899/jrheum.171007.

52. Canna SW, de Jesus AA, Gouni S, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. Nat Genet. 2014; 46:1140-6. doi: 10.1038/ng.3089.

53. Wise CA, Gillum JD, Seidman CE, et al. Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. Hum Mol Genet. 2002; 11: 961-9. doi: 10.1093/hmg/11.8.961.