Altered keratinization and vitamin D metabolism may be key pathogenetic pathways in syndromic hidradenitis suppurativa: a novel whole exome sequencing approach

Lucas Brandao[a], Ronald Moura[b], Paola Maura Tricarico[c], Rossella Gratton[c], Giovanni Genovese[d,e], Chiara Moltrasio[d], Simone Garcovich[f], Michele Boniotto[g], Sergio Crovella[c,h,1], Angelo Valerio Marzano[d,e,*,1]

[a] *Department of Pathology, Federal University of Pernambuco, Recife, Brazil*
[b] *Department of Genetics, Federal University of Pernambuco, Recife, Brazil*
[c] *Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Trieste, Italy*
[d] *Dermatology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Physiopathology and Transplantation, Università degli Studi di Milano Via Pace 9, Milan, Italy*
[e] *Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy*
[f] *Institute of Dermatology, F. Policlinico Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy*
[g] *INSERM U955 Eq. 16, Faculté de Médecine, Institut Mondor de Recherche Biomédicale and Université Paris Est- Créteil (UPEC), Créteil, France*
[h] *Department of Medical Surgical and Health Sciences, University of Trieste, Italy*

ARTICLE INFO

Accepted 1 May 2020

Keywords:
Hidradenitis suppurativa
Syndromes
Pathways
Keratinization
Vitamin D

ABSTRACT

Background: Diagnosis of pyoderma gangrenosum, acne and hidradenitis suppurativa (PASH) and pyogenic arthritis, pyoderma gangrenosum, acne, and hidradenitis suppurativa (PAPASH) patients, in spite of recently identified genetic variations, is just clinical, since most patients do not share the same mutations, and the mutations themselves are not informative of the biological pathways commonly disrupted in these patients.
Objective: To reveal genetic changes more closely related to PASH and PAPASH etiopathogenesis, identifying novel common pathways involved in these diseases.
Methods: Cohort study on PASH (n = 4) and PAPASH (n = 1) patients conducted using whole exome sequencing (WES) approach and a novel bioinformatic pipeline aimed at discovering potentially candidate genes selected from density mutations and involved in pathways relevant to the disease.
Results: WES results showed that patients presented 90 genes carrying mutations with deleterious and/or damage impact: 12 genes were in common among the 5 patients and bared 237 ns ExonVar (54 and 183 in homozygosis and heterozygosis, respectively). In the pathway enrichment analysis, only 10 genes were included, allowing us to retrieve 4 pathways shared by all patients: (1) Vitamin D metabolism, (2) keratinization, (3) formation of the cornified envelope and (4) steroid metabolism. Interestingly, all patients had vitamin D levels lower than normal, with a mean value of 10 ng/mL.
Conclusion: Our findings, through a novel strategy for analysing the genetic background of syndromic HS patients, suggested that vitamin D metabolism dysfunctions seem to be crucial in PASH and PAPASH pathogenesis. Based on low vitamin D serum levels, its supplementation is envisaged.

Abbreviations: PASH, pyoderma gangrenosum acne and hidradenitis suppurativa; PAPASH, pyoderma gangrenosum acne, pyogenic arthritis and hidradenitis suppurativa; HS, hidradenitis suppurativa; WES, whole exome sequencing; PG, pyoderma gangrenosum; PASS, pyoderma gangrenosum acne vulgaris, hidradenitis suppurativa and ankylosing spondylitis; IHS4, International Hidradenitis Suppurativa Severity Score System; DLQI, dermatology life quality index; VAS, visual analogue scale; VDR, vitamin D receptor; DAMPs, damage-associated molecular patterns; ECM, extracellular matrix; MMPss, matrix metalloproteinases.

* Corresponding author at: Dermatology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Pathophysiology and Transplantation, Università degli Studi di Milano Via Pace 9, 20122, Milan, Italy.
E-mail address: angelo.marzano@unimi.it (A.V. Marzano).
[1] Equally contributed to the article.

1. Introduction

Hidradenitis suppurativa (HS) is a chronic-relapsing inflammatory disease affecting the apocrine gland-bearing skin and presenting with pseudocystic and inflammatory nodules, abscesses and fistulas [1].

HS is usually isolated but may rarely occur also in the context of syndromes whose prototypes are pyoderma gangrenosum, acne and suppurative hidradenitis (PASH) [2–4] and pyogenic arthritis, acne, pyoderma gangrenosum, and suppurative hidradenitis (PAPASH) [5]. Genetic diagnosis of these diseases, in spite of novel variations recently identified, is just descriptive, since most cases are not sharing the same mutations, and the mutations themselves are not informative of the biological pathways commonly disrupted in PASH and PAPASH patients. As an example, the recent work of Gottlieb et al. [6] failed to reveal mutations in the genes already described in the literature (NCSTN, PSENEN, PSEN1, APH1, PSTPIP1 and NOD2) in patients suffering from autoinflammatory syndromes, in particular PASH, PsAPASH (psoriatic arthritis, pyoderma gangrenosum, acne and suppurative hidradenitis) [7] and PASS (pyoderma gangrenosum, acne, hidradenitis suppurativa and ankylosing spondylitis) [8], leading to hypothesize novel mechanisms at the basis of these conditions. To date, a main pathway associated to HS is the Notch pathway; in fact, a deficiency in Notch signaling was observed in some cases (particularly familial cases of HS), with loss of function mutations in γ-secretase genes (NCSTN, PSENEN, PSEN1 and APH1) [9]. However, the genetic variants have no functionally univocal impact on Notch expression, and HS behaves as a multifactorial disease where the altered Notch expression is not fully explaining its etiopathogenesis [10].

So, taking into account that genetic analysis, in spite of identifying mutations was not able to describe common biological traits shared by syndromic HS patients, we used a novel approach for discovering common biologic pathways in syndromic HS patients suffering from PASH (n = 4) and PAPASH (n = 1), aimed at revealing potentially causative genetic changes more closely related to the etiopathogenesis of these disorders.

2. Materials and methods

2.1. Patients

The patients followed-up were carried out at the Dermatology Unit of the University of Milan (Italy) from January 2011 to May 2019. All patients signed a written informed consent after the approval by the Area B Milan Ethics Committee (R.C 2011–2019, N.26). All patients with PASH exhibited three patterns of skin lesions: (i) Ulcers and ulcerated plaques, sometimes with vegetating aspects, consistent with PG (Fig. 1a and b); (ii) papulopustular lesions, abscesses and fistulae evolving in draining sinuses and scars, typical of HS (Fig. 1c); (iii) acne on the face (Fig.1d). The patient with PAPASH presented with the clinical triad of PASH combined with arthritis.

2.2. Genomic DNA extraction and whole exome sequencing

Genomic DNAs have been extracted from saliva using the Oragene-DNA (Ottawa, Canada) kit following the manufacturer's protocols. After DNA quantity and quality check by agarose gel and Qubit assay (Invitrogen, Oregon USA), whole exome sequencing (WES) with 100X of expected coverage has been performed in outsourcing by Macrogen (Seoul, Korea). In brief, DNA Exome Sequencing reactions were performed through Illumina® HiSeq 2500 System, after the library preparation (SureSelect Human all exons V6 kit).

2.3. Data analysis

The global coverage was re-calculated through Picard tools and retrieved an average of 93.9%, 36.0% and 9.3% for 10, 50, 100x coverage, respectively. Adapters were trimmed using the Trim Galore, searching and removing Illumina adapters, reads with length below 15 base pairs, and low-quality ends from reads with Phred33 score below 20 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/).

The FASTQ file was aligned using Burrows-Wheeler Aligner (BWA) Software Package [11], specifically the bwa-mem tool, with the Human Genome version 38 (GRCh.38) as reference. Then, Picard tools (https://broadinstitute.github.io/picard/) was used to mark and remove duplicates reads; GATK v. 4.1.2.0 (https://software.broadinstitute.org/gatk/) allowed base recalibration and variant calling, excluding those variants with low mapping and genotyping quality (MQ < 40 and GQ < 20, respectively). Quality control and assurance were visualized by fastQC.

ANNOVAR software [12] was used for variant annotation based on databases relative to the GRCh.38 reference genome (dbSNP 151, CADD; GERP++, SIFT, PolyPhen2, FATHMM, COSMIC70, ClinVar, 1000 Genomes Project, ExAC 03, genomicsSuperDups, wgRNA, GWAS Catalog, and Interpro).

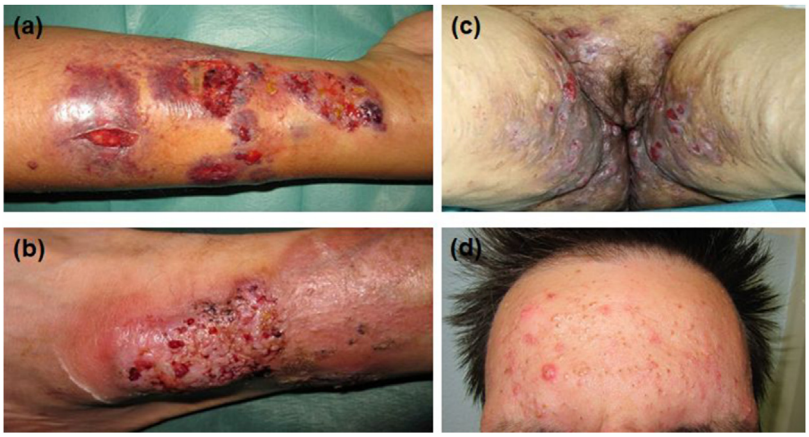


Fig. 1. Pyoderma, acne and suppurative hidradenitis (PASH) syndrome. (a) Acne consisting of papules, pustules and comedones on the forehead; (b) ulcerated nodules of hidradenitis suppurativa coalescing into infiltrated plaques on the genitalia; (c) ulcerative lesions of pyoderma gangrenosum on the arm; (d) pyoderma gangrenosum manifesting as ulcer with vegetating features.

A novel homemade framework was customized, through R Software Environment, to analyze the annotated exome content and subsequently to unravel pathways involvement in PASH and PAPASH phenotypes. Basically, the investigation focused on variants located in the coding regions (ExonVar). Instead of searching only known or novel mutations, the framework outputted the distribution, density, PolyPhen, SIFT, FATHMM description of ExonVar, including single nucleotide polymorphism (SNP) and insertion/deletion (indel) for each patient. Then, the pathway prediction was performed employing two strategies: (1) Raking common genes with the higher score in the three proteins functional algorithm prediction (2) including all genes carrying, at least one, ExonVar in a pathway enrichment analysis (PEA) using the Reactome database, (https://reactome.org/) from ReactomePA R package. Pathways with adjusted p-value < 0.05 were included for further analyzes. Next, pathways shared by all five patients, as well as exclusive pathways, were visualized through Venn Diagram. To exploit the overall ExonVar impact, the median HumDiv PolyPhen, SIFT and FATHHM score was established for each shared pathway by calculating the mean of nonsynonymous (ns) ExonVar values retrieved with PolyPhen analysis.

3. Results

3.1. Patients' clinical features

The present case series included 5 patients, 4 of whom with PASH and the other one with PAPASH syndrome. Mean age of onset of HS was 20.2 (range, 15–32) years, with mean diagnostic delay of 3 years. The most frequently involved sites were perianal area (n = 3) and axillae (n = 3), followed by inguinal region (n = 2), genitalia (n = 2), neck (n = 1), back (n = 1), gluteal region (n = 1) and submammary folds (n = 1). Family history was negative for HS in all patients. Among the aggravating factors, sweating was the most common (n = 4), followed by obesity (n = 2) and seasonal worsening (n = 2), while smoking was reported only in a patient. Premenstrual exacerbation was observed in 1 out of 2 female patients. Pilonidal cyst and bariatric surgery were recorded in two patients, respectively. All patients were in Hurley stage III, with a mean International Hidradenitis Suppurativa Severity Score System (IHS4) [13] of 16.8 (range, 12–34), a mean Dermatology Life Quality Index (DLQI) [14] of 22.4 (range, 15–27) and a mean pain Visual Analogue Scale (VAS) [15] of 6.6 (range, 3–8). Mean age at onset of pyoderma gangrenosum (PG) was 23.8 (range, 18–33) years. Ulcerative lesions were present in all cases and associated with vegetating features in one patient; the disease was widespread in three cases. The most frequently involved sites were lower limbs (n = 3), followed by presternal region, (n = 1), back (n = 1) and perianal region (n = 1). Neither family history of PG nor extracutaneous involvement were recorded. All patients had, as common characteristic, vitamin D levels lower than normal, with a mean value of 10 ng/ml (range: 8–15).

Mean age at onset of acne was 17.8 (13–33) years. Acne lesions involved the face in all cases and also the trunk in two of them.

The most relevant comorbidities were inflammatory bowel disease (n = 1) and psoriasis (n = 1). History of arthritis involving the wrists and ankles was recorded only in the patient with PAPASH. An increase in acute phase reactants was seen in all patients, with mean C reactive protein of 22.14 mg/dl (range, 7.5–43.7) and erythrocyte sedimentation rate of 38.9 mm/hr (range, 12–68). Mean hemoglobin value resulted 11.1 g/dl and mean gamma globulins value was 1900 mg/dl. Two patients had anemia and three showed polyclonal hypergammaglobulinemia. Before our observation, all patients underwent various cycles of systemic and topical antibiotics, without benefit. Cyclosporine A had been given in two patients, dapsone in one and isotretinoin in another one, also without clinical response. In all subjects, one of the following biologic agents was administered at our department: adalimumab (n = 2), infliximab (n = 2), and ustekinumab (n = 1), with a good control of the cutaneous picture. Ustekinumab was given in one patient as switch over from infliximab due to loss of efficacy.

3.2. Genetic analysis

Exome genotyping of the 5 patients included in our study revealed, in overall analysis, 320,533 different variants with an average of 132,788 individual variations in each patient. Most of them were distributed on chromosome 119 and 2 (mean of 11281, 8924, 8607, respectively). Exonic changes (ExonVar) represented 13.98% (44,815) of all mutations and were distributed along 13,170 genes (Fig. 2a).

Around 48% of ExonVar had a possible impact on peptides/proteins. The nonsynonymous (ns) ExonVar were the most frequent (47.2%), affecting 8843 different genes (Table 1). Only these genes and ns ExonVar were considered for subsequent analysis.

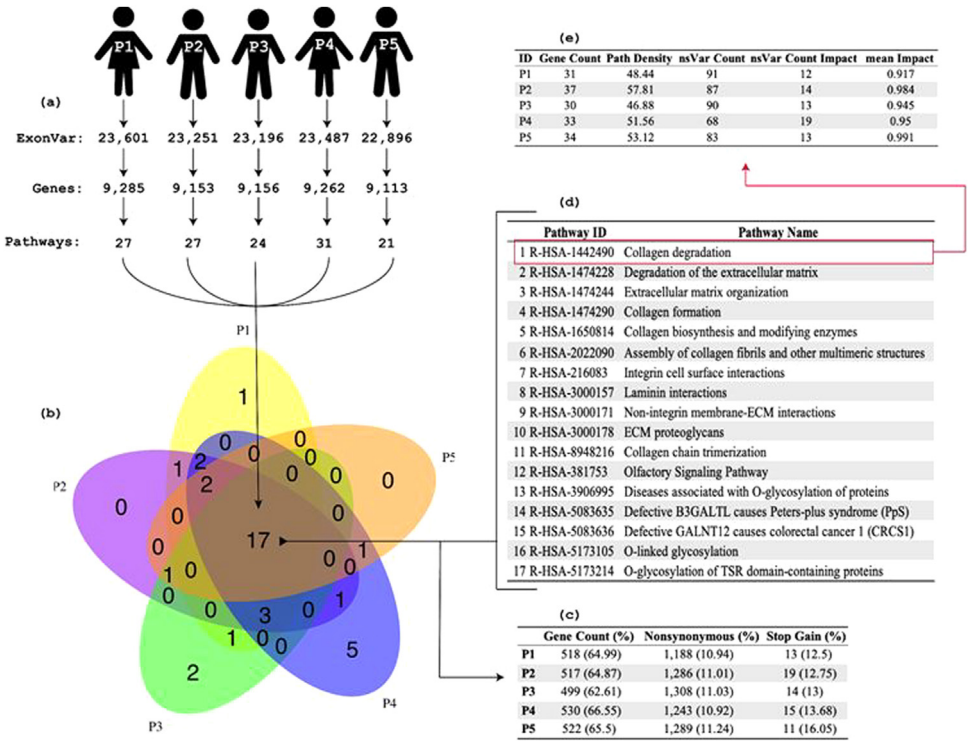
To unravel ExonVar, genes and pathways involvement in PASH and PAPASH patients two strategies were established. First, we ranked the genes according to variant density and median score of three variant impact prediction: HumDiv PolyPhen2 (Polymorphism Phenotyping v2), SIFT (sorting intolerant from tolerant) and FATHMM (Functional Analysis through Hidden Markov Models). Patients presented 90 genes carrying mutations with deleterious and/or damage impact; within these 90 genes, 12 were in common among the 5 patients and bared 237 nsExonVar (54 and 183 in homozygosis and heterozygosis, respectively). In the pathway enrichment analysis (PEA), only 10 genes were included, allowing us to retrieve 4 pathways shared by all patients: (1) Vitamin D (calciferol) metabolism, (2) keratinization, (3) formation of the cornified envelope and (4) metabolism of steroids. Then, we expanded the analysis to check how these four pathways were altered by all functional mutations in each patient (Table 2). As an example, about 45% of genes on Vitamin D pathway carry at least one ExonVar; up to 7 variations impact the protein function, showing that this pathway suffers mutations, and its function could be diminished or impaired.

A second approach focused on a global ExonVar overview. All genes bearing ExonVar from each patient were filtered and included in PEA to depict an individual mutational pathway landscape (not shown). Venn diagram analysis allowed us to identify 17 common pathways shared by all patients (Fig. 2b). 797 genes took part in these 17 shared pathways and 65% of these genes presented ExonVar (mean of 519 genes), Fig. 2c. Around 11% of all nsExonVar impacted these 17 shared pathways with mean value higher than 0.94 in HumDiv PolyPhen score. Additionally, more than 10% of all other mutations present a functional variant impact. As listed in Fig. 2d the first 11 pathways are all related to biological processes involving the skin such as collagen and extracellular matrix remodeling). Fig. 2e shows an example of how the density variation and polyPhen impact the collagen degradation pathway.

4. Discussion

Our findings indicate that the mainly affected pathway in PASH and PAPASH patients was vitamin D metabolism (Table 2), followed by the one involved in keratinization.

Vitamin D has been predicted as able to regulate skin homeostasis by controlling proliferation and differentiation of epidermis and adnexal structures, particularly hair follicle [16]. Moreover, vitamin D contributes to the regulation of the immune response at the skin level, counteracting inflammation [17]. All



(e)

ID	Gene Count	Path Density	nsVar Count	nsVar Count Impact	mean Impact
P1	31	48.44	91	12	0.917
P2	37	57.81	87	14	0.984
P3	30	46.88	90	13	0.945
P4	33	51.56	68	19	0.95
P5	34	53.12	83	13	0.991

(d)

	Pathway ID	Pathway Name
1	R-HSA-1442490	Collagen degradation
2	R-HSA-1474228	Degradation of the extracellular matrix
3	R-HSA-1474244	Extracellular matrix organization
4	R-HSA-1474290	Collagen formation
5	R-HSA-1650814	Collagen biosynthesis and modifying enzymes
6	R-HSA-2022090	Assembly of collagen fibrils and other multimeric structures
7	R-HSA-216083	Integrin cell surface interactions
8	R-HSA-3000157	Laminin interactions
9	R-HSA-3000171	Non-integrin membrane-ECM interactions
10	R-HSA-3000178	ECM proteoglycans
11	R-HSA-8948216	Collagen chain trimerization
12	R-HSA-381753	Olfactory Signaling Pathway
13	R-HSA-3906995	Diseases associated with O-glycosylation of proteins
14	R-HSA-5083635	Defective B3GALTL causes Peters-plus syndrome (PpS)
15	R-HSA-5083636	Defective GALNT12 causes colorectal cancer 1 (CRCS1)
16	R-HSA-5173105	O-linked glycosylation
17	R-HSA-5173214	O-glycosylation of TSR domain-containing proteins

(c)

	Gene Count (%)	Nonsynonymous (%)	Stop Gain (%)
P1	518 (64.99)	1,188 (10.94)	13 (12.5)
P2	517 (64.87)	1,286 (11.01)	19 (12.75)
P3	499 (62.61)	1,308 (11.03)	14 (13)
P4	530 (66.55)	1,243 (10.92)	15 (13.68)
P5	522 (65.5)	1,289 (11.24)	11 (16.05)

Fig. 2. Exome analysis for the PASH and PAPASH patients. In (a) are summarized the counts of exonic variants (ExonVar), the number of genes bearing these nonsynonymous mutations and the pathways in which they are involved. The Venn diagram (b) intersects 17 common pathways found among the five patients. In (c), we can see a table with the number of genes, nonsynonymous and stop gain variants associated to the common pathways (d). Finally, in (e) it is displayed genetic information regarding the Collagen degradation pathway.

Table 1
Count and classification of the exonic variants found among PASH and PAPASH patients.

Exon function	P1	P2	P3	P4	P5	All
Nonsynonymous SNV	10862	10787	10768	10875	10569	21175
Insertion/Deletion Frameshift (n)	102/141 (243)	109/144 (253)	95/160 (255)	92/123 (215)	97/132 (229)	216/305 (521)
Stop Gain/Loss (n)	104/15 (119)	102/14 (116)	100/13 (113)	95/10 (105)	81/9 (90)	231/2 (233)
Insertion/Deletion nonframeshift (n)	174/207 (381)	151/203 (354)	170/211 (381)	168/211 (379)	158/198 (356)	342/438 (780)
Synonymous SNV	11798	11545	11446	11698	11388	21670
Unknown	213	207	245	228	272	469

Table 2
List of the most significant pathways, by ReactomePA, associated to the variants with a functional impact to its proteins.

Path (ID) [n]	ID	Gene Count[a]	Pathway Density (%)	nsExonVar Count				Median impact			
				All	PolyPhen2	SIFT	FATHMM	PolyPhen2[b]	SIFT	FATHMM	
Vitamin D	P1	5	45.45	20	5	7		7	0.996	0.005	−2.880
-calciferol-metabolism	P2	5	45.45	15	3	4	4	0.993	0.015	−2.880	
(R-HSA-196791)	P3	4	36.36	13	3	5	5	0.993	0.020	−2.880	
[11]	P4	5	45.45	15	4	4	4	0.995	0.015	−2.880	
	P5	4	36.36	12	2	3	5	0.993	0.020	−2.840	
Keratinization	P1	102	47.66	196	32	49		53	0.982	0.010	−2.373
(R-HSA-6805567)	P2	107	50.00	241	39	48	63	0.975	0.010	−2.380	
[214]	P3	97	45.33	192	32	49	68	0.952	0.010	−2.375	
	P4	100	46.73	224	31	43	49	0.924	0.010	−2.405	
	P5	91	42.52	170	30	36	44	0.983	0.020	−2.410	
Formation of the Cornified	P1	67	51.94	128	24	37		53	0.988	0.010	−2.373
envelope R-HSA-6809371 [129]	P2	70	54.26	179	32	38	63	0.975	0.012	−2.380	
	P3	62	48.06	132	23	38	68	0.928	0.010	−2.375	
	P4	62	48.06	151	23	30	49	0.924	0.010	−2.405	
	P5	56	43.41	102	18	24	44	0.988	0.020	−2.410	
Metabolism	P1	46	30.67	77	12	12		18	0.970	0.010	−2.510
of steroids	P2	38	25.33	70	11	13	16	0.970	0.015	−2.785	
R-HSA-8957322	P3	44	29.33	68	7	9	16	0.970	0.025	−2.625	
[150]	P4	41	27.33	66	9	10	12	0.993	0.022	−2.785	
	P5	38	25.33	63	8	8	15	0.982	0.025	−2.840	

[a] Number of genes carrying a nonsynonymous Exon Variant (nsExonVar).
[b] HumDiv score.

patients of our study presented high levels of variant density in vitamin D pathway, some of them with high deleterious and damage impact. We confirmed this result by analysing the vitamin D serum levels in our patients, observing low levels of vitamin D in all probands. Therefore, these findings evoked the idea that vitamin D insufficiency could be involved in PASH and PAPASH pathogenesis.

To date, vitamin D levels had not been analyzed in syndromic HS, but some studies have been describing its levels and inflammatory role in HS or acne, both present in syndromic HS [18–20].

Kelly et al. [18] described 12 out of 16 HS patients with low vitamin D levels; in addition, Guillet et al. [20] reported that 22 patients (100% of their case series) had vitamin D deficiency (serum level <30 ng/mL), 36% of whom were severely deficient (serum level <10 ng/mL). Lim et al. [19] observed, in a case-control study, that 48.8% of patients with acne presented vitamin D deficiency, that was found in only 22.5% of the healthy controls. In this study, vitamin D levels were inversely correlated to the severity of acne and to the number of inflammatory lesions.

In all the three studies mentioned above, supplementation of vitamin D induced a reduction in the number of inflammatory lesions while no change was observed in non-inflammatory lesions neither of acne nor of HS. Moreover, a recent systematic review indicated a Grade B of recommendation for vitamin D supplementation for both skin disorders, with a level of evidence IIb and III for acne and HS, respectively [17]. We are aware that hypovitaminosis D may depend also on obesity, smoking and inflammatory bowel diseases, all clinical and behavioral conditions associated with HS and its syndromic forms.

A knockout homozygous mouse model for Vitamin D Receptor (VDR) revealed dilation of hair follicles lacking hair shafts and exhibiting thin hair sheaths, with formation of dermal cysts containing tissue debris [21].The changes seen in this experimental model are closely similar to the early-phase histopathological pattern of HS, consisting of dilation and disruption of hair follicles with dermal cyst formation [22].

VDR knockout mice also showed a remarked decrease in the expression of proteins that take part in the formation of cornified envelope and keratinization, such as involucrin, profilaggrin and loricrin [21].

Interestingly, vitamin D metabolism and keratinization pathways presented high variant density in our 5 patients. Our data corroborate the recent view of HS as an autoinflammatory keratinization disease [23] and are in line with the preliminary studies of our group that reported in syndromic HS a number of mutations involving different genes related to classic monogenic autoinflammatory diseases [3,4,24].

The keratinization process comprehends 8 complex steps as described in the Reactome database (https://reactome.org/PathwayBrowser/#/R-HSA-6805567&SEL = R-HSA-6814374&PATH = R-HSA-1266738) and includes the formation of a cornified envelope. These 8 routes are able to maintain the mechanical stability of individual cells and epithelial tissues [25] by bundling the keratin filaments into tonofilaments that span the cytoplasm and bind to desmosomes and other cell membrane structures [26]. Two hundred and fourteen genes are involved in this function, regulating keratin production, maturation and degradation.

The dysregulation of keratinization has been associated with several skin diseases, among which psoriasis is the paradigma [27] and hyperkeratinization of the terminal portion of the hair follicle is regarded as the *primum movens* in HS pathogenesis [28,29]. Hyperkeratinization and hyperplasia of the follicular epithelium promotes accumulation of keratin-rich material with follicular occlusion and dilatation, finally leading to hair follicle rupture. It has been suggested that this keratin-rich material together with the disrupted follicle products act as damage-associated molecular patterns (DAMPs), triggering autoinflammation [29,30].

In conclusion, we described altered shared biological pathways in PASH and PAPASH patients, particularly keratinization and vitamin D metabolism. We are aware that our pathway analysis is just based on WES findings, needing a further functional confirmation; however, at least for vitamin D pathway, we have found confirmation in its low levels seen in all our patients.

Additional contributions

None

Funding resources

European Community EraPerMed grant 2018/137,Project Ricerca Corrente RC16/18 from IRCCS Burlo Garofolo, Project ISE-EMH Intereg ITA-SLO 2019,Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – 4303553/2018-9

Declaration of Competing Interest

The authors have no conflict of interest to declare

References

[1] G.B. Jemec, Clinical practice. Hidradenitis suppurativa, N. Engl. J. Med. 366 (January 2) (2012) 158–164.

[2] M. Braun-Falco, O. Kovnerystyy, P. Lohse, T. Ruzicka, Pyoderma Gangrenosum, Acne, and Suppurative Hidradenitis (PASH)-a New Autoinflammatory Syndrome Distinct from PAPA Syndrome, J. Am. Acad. Dermatol. 66 (3) (2012) 409–415.

[3] A.V. Marzano, I. Ceccherini, M. Gattorno, D. Fanoni, F. Caroli, M. Rusmini, et al., Association of pyoderma gangrenosum, acne, and Suppurative Hidradenitis (PASH) shares genetic and cytokine profiles with other autoinflammatory diseases, Medicine (Baltimore) 93 (27) (2014) e187.

[4] A.V. Marzano, G. Damiani, I. Ceccherini, E. Berti, M. Gattorno, M. Cugno, Autoinflammation in pyoderma gangrenosum and its syndromic form (pyoderma gangrenosum, acne and suppurative hidradenitis), Br. J. Dermatol. 176 (6) (2017) 1588–1598.

[5] A.V. Marzano, V. Trevisan, M. Gattorno, I. Ceccherini, C. De Simone, C. Crosti, Pyogenic arthritis, pyoderma gangrenosum, acne, and hidradenitis suppurativa (PAPASH): a new autoinflammatory syndrome associated with a novel mutation of the PSTPIP1 gene, JAMA Dermatol. 149 (6) (2013) 762–764.

[6] J. Gottlieb, M. Madrange, C. Gardair, E. Sbidian, A. Frazier, P. Wolkenstein, et al., PAPASH, PsAPASH and PASS autoinflammatory syndromes: phenotypic heterogeneity, common biological signature and response to immunosuppressive regimens, Br. J. Dermatol. 181 (4) (2019) 866–869.

[7] R. Saraceno, G. Babino, A. Chiricozzi, A. Zangrilli, S. Chimenti, PsAPASH: a new syndrome associated with hidradenitis suppurativa with response to tumor necrosis factor inhibition, J. Am. Acad. Dermatol. 72 (January (1)) (2015) e42–4.

[8] M. Leuenberger, J. Berner, J. Di Lucca, L. Fisher, N. Kaparos, C. Conrad, et al., PASS syndrome: an IL-1-Driven autoinflammatory disease, Dermatology 232 (2) (2016) 254–258.

[9] X. Xiao, Y. He, C. Li, X. Zhang, H. Xu, B. Wang, Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through the Notch and phosphoinositide 3-kinase/AKT signalling pathways, Br. J. Dermatol. 174 (2016) 522–532.

[10] J.W. Frew, K. Navrazhina, In silico analysis of gamma-secretase-Complex mutations in hidradenitis suppurativa demonstrates disease-specific substrate recognition and cleavage alterations, Front Med. (Lausanne). 6 (2019) 206.

[11] H. Li, R. Durbin, Fast and accurate long-read alignment with Burrows-Wheeler Transform, Bioinformatics 26 (5) (2010) 589–595.

[12] K. Wang, M. Li, H. Hakonarson, ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data, Nucleic Acids Res. 38 (16) (2010) e164.

[13] C.C. Zouboulis, T. Tzellos, A. Kyrgidis, G.B.E. Jemec, F.G. Bechara, E.J. Giamarellos-Bourboulis, et al., European Hidradenitis Suppurativa Foundation Investigator Group. Development and validation of the International Hidradenitis Suppurativa Severity Score System (IHS4), a novel dynamic scoring system to assess HS severity, Br. J. Dermatol. 177 (2017) 1401–1409.

[14] A.Y. Finlay, G.K. Khan, Dermatology Life Quality Index (DLQI)–a simple practical measure for routine clinical use, Clin. Exp. Dermatol. 19 (1994) 210–216.

[15] D.D. Price, P.A. McGrath, A. Rafii, B. Buckingham, The validation of visual analogue scales as ratio scale measures for chronic and experimental pain, Pain 17 (1983) 45–56.

[16] E. Kechichian, K. Ezzedine, Vitamin D. And the skin: an. Update for dermatologists, Am. J. Clin. Dermatol. 19 (2) (2018) 223–235.
[17] F.J. Navarro-Triviño, S. Arias-Santiago, Y. Gilaberte-Calzada, Vitamin D. And the skin: A. Review for dermatologists, Actas Dermosifiliogr. 110 (4) (2019) 262–272.
[18] G. Kelly, C.M. Sweeney, R. Fitzgerald, M.P. O'Keane, M. Kilbane, A. Lally, A.M. Tobin, M.J. McKenna, B. Kirby, Vitamin D status in hidradenitis suppurativa, Br. J. Dermatol. 170 (2014) 1379–1380.
[19] S.K. Lim, J.M. Ha, Y.H. Lee, Y. Lee, Y.J. Seo, C.D. Kim, et al., Comparison of vitamin d levels in patients with and without acne: a case-control study combined with a randomized controlled trial, PLoS One 11 (August 8) (2016).
[20] A. Guillet, A. Brocard, K. Bach Ngohou, N. Graveline, A.G. Leloup, D. Ali, et al., Verneuil's disease, innate immunity and vitamin d: a pilot study, J. Eur. Acad. Dermatol. Venereol. 29 (7) (2015) 1347–1353.
[21] Z. Xie, L. Komuves, Q.C. Yu, H. Elalieh, D.C. Ng, C. Leary, et al., Lack of the vitamin d receptor is associated with reduced epidermal differentiation and hair follicle growth, J. Invest. Dermatol. 118 (1) (2002) 11–16.
[22] C.-W.C. Yu, M.G. Cook, Hidradenitis suppurativa: a disease of follicular epithelium, rather than apocrine glands, Br. J. Dermatol. 112 (6) (1990) 763–769.
[23] T. Takeichi, T. Matsumoto, T. Nomura, M. Takeda, H. Niwa, M. Kono, et al., A novel NCSTN missense mutation in the signal peptide domain causes hidradenitis suppurativa, which has features characteristic of an autoinflammatory keratinization disease, Br. J. Derm. (2019).
[24] P.M. Tricarico, M. Boniotto, G. Genovese, C. Zouboulis, A.V. Marzano, S. Crovella, An integrated approach to unravel hidradenitis suppurativa etiopathogenesis, Front. Immunol. 10 (2019) 892.
[25] R. Moll, M. Divo, L. Langbein, The human keratins: biology and pathology, Histochem. Cell Biol. 129 (6) (2008) 705–733.
[26] J. Waschke, The desmosome and pemphigus, Histochem. Cell Biol. 130 (1) (2008) 21–54.
[27] C. Albanesi, S. Madonna, P. Gisondi, G. Girolomi, The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis, Front. Immunol. 9 (2018) 1549.
[28] M. von Laffert, P. Helmbold, J. Wohlrab, E. Fiedler, V. Stadie, W.C. Marsch, Hidradenitis suppurativa (Acne inversa): early inflammatory events at terminal follicles and at interfollicular epidermis*, Exp. Dermatol. 19 (6) (2010) 533–537.
[29] B. Melnik, G. Plewig, Are disturbances of omega-6-fatty acid metabolism involved in the pathogenesis of atopic dermatitis? Acta Derm. Venereol. Suppl. 176 (1992) 77–85.
[30] D.L. Kastner, I. Aksentijevich, R. Goldbach-Mansky, Autoinflammatory disease reloaded: a clinical perspective, Cell. 140 (6) (2010) 784–790.