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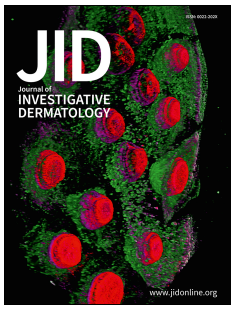

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A20 haploinsufficiency: A systematic review of 177 cases.

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Abstract

A20 haploinsufficiency (HA20) is an autoinflammatory disease caused by defective inactivation of NF-κB pathway. We conducted a systematic literature review of articles reporting patients with *TNFAIP3* mutations from 2016 to August 2023 following PRISMA guidelines. Data of 177 patients from 65 articles were retrieved (108 women). The principal features were: mucosal ulcers (n=129), fever (n=93) followed by gastrointestinal (n=81), skin features (n=76), autoimmunity (n=61) including thyroiditis (n=25) and Lupus (n=16), and joint involvements (n=54). Five patients had died at the time of publication. In 54/63 patients, C-reactive protein was significantly elevated during flares, with a median of 51mg/L. Most commonly used treatment included corticosteroids and non-steroidal anti-inflammatory drugs (n=32), TNF-blockers (n=29), colchicine (n=28) and methotrexate (n=14). *TNFAIP3* variants impacted the OTU domain in 92 cases and a Zinc finger domain in 68 cases. Geographic origin, gender and variant type significantly impacted phenotype. A better understanding of the wide HA20 phenotype could facilitate the diagnosis process. Much remains to be elucidated about pathogenesis and treatment to improve outcome in HA20 patients.

Introduction

A20 haploinsufficiency (HA20) is an inborn error of immunity caused by heterozygous loss-of-function mutations in the highly conserved gene *TNFAIP3*, which encodes the protein A20 (Yu et al. 2020). In humans, *TNFAIP3* was identified in genome-wide association studies long before the discovery of HA20, as single-nucleotide polymorphisms were associated with the development of autoimmune diseases such as Systemic Lupus Erythematous (SLE), Rheumatoid arthritis (RA) and diabetes (Vereecke et al. 2014). A monogenic *TNFAIP3*-associated disease was described in 2016 upon the description of patients with heterozygous loss-of-function mutations in this gene. Since then, the analysis of *TNFAIP3* has been added to next generation sequencing (NGS) panels worldwide, allowing the diagnosis and publication of hundreds new cases. These publications have enlarged the clinical spectrum of HA20, which has finally evolved to be considered as a distinct entity at the crossroads between auto-inflammation and autoimmunity (Yu et al. 2020).

A20 is a 790 amino-acids protein with 8-domains consisting of an N-terminal ovarian tumor (out) and a C-terminal region containing 7 zinc-finger motifs (Chen et al. 2020b). A20 possesses unique ubiquitin-editing properties allowing both ubiquitination and deubiquitylation. By tagging important actors of canonical NF-κB activation (RIP1, TRAF6 among others) for degradation to the proteasome and by removing activating K63-ubiquitin from other proteins of the pathway such as NEMO (IKKγ), A20 acts as a negative regulator of inflammation in a negative regulatory loop. It also suppresses the activation of the MAP kinases (MAPK) and downregulate the activation of the NLRP3 inflammasome (Catrysse et al. 2014). Therefore, the systemic inflammatory manifestations of HA20 result from the insufficient suppression of NF-κB, MAPK and NLRP3 activity in immune cells, especially in myeloid cells. A20 is also abundantly expressed in B cells, where it inhibits the NF-κB pathway, and raises the signaling threshold required for B cell proliferation and survival (Das et al. 2018). As a result, A20-deficient B cells are hyper-responsive to activating stimuli and susceptible to loss of tolerance and to develop autoimmune diseases (Das et al. 2018).

To date, the phenotypic spectrum of HA20 remains unsettled and the understanding of pathogenesis incomplete. Our objective was to summarize the current knowledge on the genetic, pathophysiology and clinical features of HA20 through a systematic literature review since the description of the disease.

Results

1/ Genetics and pathogenesis

Eighty-four different variants have been described including premature truncating codon and missense variants (Figure 1.A). A large proportion of the 177 patients had variants impacting the N-terminal OTU domain (n=92 patients, 52.3%), 47.8% of them being stop-gained (n=44) and 28.3% frameshift mutants (n=26). Other patients had variants lying in the C-terminal domain of A20, composed of 7 zinc-finger domains, (n=68 patients, 38.4 %), with mostly stop-gain, frameshift or splice variants (69.1%) (Supplementary table 2). A deletion of the whole *TNFAIP3* gene was described in 8 patients, including 7 for which deletion comprised surrounding genes, ranging from 236kb to 13.3MB. Two patients inherited HA20 from a parent with low-frequency gonadal mosaicism. Both were asymptomatic and the frequency of mutant allele was 10.06% and 16.7% (Kadowaki et al. 2018). Five patients displayed additional variants in other genes: 3 displayed a class IV *MEFV*, one unclassified IL-36RN variant, and one patient a complex genotype with variants in VPS13B, PIK3R1 and NFAT5 (Horita et al. 2019; Liang et al. 2019; Niwano et al. 2022). Of note, in this cohort, only 23.2% of the variants have been tested in an ectopic expression system, 47.6% of the missense variants and 15.6% of the mutations giving rise to an early stop codon (Supplementary Table 3).

2/ Epidemiology

HA20 was first described in 2016 as a new monogenic Behcet's disease (BD) and has finally evolved to be considered as a distinct entity. As of August 2023, after eliminating duplicates, 177 cases with sufficient clinical data to be included in the review were identified. (Figure 1.B). Ethnicity was described in 26 patients (14%) including Chinese Han (n=16), Hispanic patients (n=3), Turk (n=2), African American, Ashkenazi (n=1, each). Country of origin was indicated in 51 patients (Great Britain, Japan (n=27), China (n=15), Italia (n=4), Turkey (n=3), Pakistani/Indian (n=2), Great-Britain and Spain (n=1, each)). Finally, eastern European origin was suggested in 26 patients (Caucasian (n=14), European American (n=10), White (n=2)). Information was incomplete for 74 patients (40%). We classified patients in 4 categories: West Asia (Turkish and Saudi patients, n=6), East Asia (Chinese, Chinese Han, Japanese patients, n=58) and Europe (Caucasian, European American, White patients, n=32), and South Asia (Pakistani/Indian patients, n=2). Statistical analyses compared group with ≥5 patients (supplementary table 3). Patients from West Asia were 2-3 times more likely to suffer from

genital ulcers (p=0.03), autoimmunity and thyroiditis (p<0.001) than their counterparts. Moreover, Skin features were more frequent in European patients. Finally, patients from West Asia presented with fever twice as frequently than both those from Europe and East Asia (p<0.01).

Female to male ratio in published patients is 1.1:1 (108 women, 94 men). First symptoms occurred in childhood or teenage years in 132/141 patients (94%) with a median age of 3 years old [0-17]. HA20 debuted in adulthood in 9 patients, with a median age of 20 years old [18-35] (Duan et al. 2019; Harris et al. 2018; He et al. 2020; Horita et al. 2019; Niwano et al. 2022; Tian et al. 2022).

3/ Clinical features (Table 1 and Figure 1.c)

Overall, HA20 is a multi-systemic disease. In 27 patients, Mucosal ulcers (including 9 with genital ulcer), fever and/or lymphadenopathies were the only symptoms. Besides, 46 Patients exhibited a single organ involvement. Finally, more than one organ was affected in 100 patients, in addition to general and mucosal features.

A. Fever

Half of HA20 patients exhibit fever during the course of the disease (n=93). In most cases (n=80), it consisted of periodic episodes of fever, which lasted for a median duration of 5 days. The duration is variable and goes from 24 hours to 14 days. Fever attacks were not cyclic and may occur several times a month or once a year. In 16 patients, the disease manifested as a protracted fever that required anti-inflammatory treatment to resolve (Aslani et al. 2022; Jo et al. 2022; Kadowaki et al. 2018; Kim et al. 2020; Lawless et al. 2018; Li et al. 2019; Liu et al. 2023; Ohnishi et al. 2017; Shaheen et al. 2021; Tian et al. 2022; Tsuchida et al. 2019). These 2 types of fever episodes may coexist in the same patient (Jo et al. 2022; Kadowaki et al. 2018; Kim et al. 2020; Lawless et al. 2018; Shaheen et al. 2021; Tian et al. 2022; Tsuchida et al. 2019).

B. Mucosal ulcers

Mucosal ulcers are a hallmark of the disease and were present in 129 patients (73%). They can appear with or without inflammatory/febrile episodes. Mouth ulcers resemble common benign ulcers, although extensive stomatitis have been reported. In one of the first description,

Aeschlimann *et al* described scarring mouth ulcers in a cohort of 16 patients, however it was never described afterwards. Therefore, scarring ulcers does not seem to be a necessary feature of HA20.

More than a third of HA20 patients have displayed genital ulcers during the course of the disease (n=63), which can occur as early as in infancy. They are significantly more frequent in females than in males (48 vs 15 patients, p=0.004). Precise localization and characteristics were not described. Genital ulcerations have been described in 6 patients without mouth ulcers.

C. Gastrointestinal manifestations

Gastrointestinal involvements affected 81 patients (46%). They include abdominal pain (48 patients), diarrhea (41 patients) and bloody stool (21 patients). Two main phenotypes emerge from gut involvements: 29 patients exhibited isolated or multiple GI ulcers (Aeschlimann et al. 2018; Berteau et al. 2019; Chen et al. 2020a; Deshayes et al. 2021; Dong et al. 2019; Dong et al. 2019; Duncan et al. 2018; El Khouri et al. 2023; He et al. 2020; Jiang et al. 2022; Kadowaki et al. 2021a; Kadowaki et al. 2018; Li et al. 2019; Liang et al. 2019; Liang et al. 2019; Mitsunaga et al. 2022; Ohnishi et al. 2017; Sato et al. 2018; Shimizu et al. 2020; Suri et al. 2021; Taniguchi et al. 2021; Tian et al. 2022; Tsuchida et al. 2019; Uchida et al. 2020; Wakatsuki et al. 2023; Yan et al. 2021; Zanatta et al. 2022; Zhang et al. 2022; Zhang et al. 2022; Zheng et al. 2018; Zou et al. 2020), while 17 patients displayed definite colitis, including 14 who also presented GI ulcers (Chen et al. 2020a; He et al. 2020; Kadowaki et al. 2018; Shiraki et al. 2021b; Suri et al. 2021; Taniguchi et al. 2021; Tsuchida et al. 2019; Uchida et al. 2020; Yan et al. 2021; Ye et al. 2017; Zanatta et al. 2022; Zheng et al. 2018; Zou et al. 2020). This phenotype occurs in significantly younger patients (6 months vs 3 years, p=0.02). It developed before the age of 6 in all but one patient and can therefore be classified as Very-Early Onset Inflammatory Bowel Disease. The whole gastrointestinal tract can be affected by ulcers and were described in the colon in 22 cases (Chen et al. 2020a; Dong et al. 2019; El Khouri et al. 2023; He et al. 2020; Jiang et al. 2022; Mitsunaga et al. 2022; Ohnishi et al. 2017; Shimizu et al. 2020; Suri et al. 2021; Taniguchi et al. 2021; Tian et al. 2022; Tsuchida et al. 2019; Uchida et al. 2020; Wakatsuki et al. 2023; Yan et al. 2021; Zanatta et al. 2022; Zhang et al. 2022; Zheng et al. 2018), the stomach and/or duodenum in 9 cases (Dong et al. 2019; Sato et al. 2018; Tian et al. 2022; Tsuchida et al. 2019; Zheng et al. 2018) and the small bowel in 6 cases (Deshayes et al. 2021; He et al. 2020; Kadowaki et al. 2021a; Suri et al. 2021; Tian et al. 2022). In 4 patients,

ulcers were restricted to the upper gastrointestinal tract (Dong et al. 2019; Kadowaki et al. 2021a; Ohnishi et al. 2017; Sato et al. 2018; Zhang et al. 2022). There can be findings of gastritis (4 patients). Endoscopic findings of patients with colitis could be compatible with ulcerative colitis (UC) in 2 patients with rectal and descending colon ulcers (Kadowaki et al. 2018; Taniguchi et al. 2021). Moreover, 6 other patients exhibited histological features that could suggest UC, including crypt atrophy, cryptitis, neutrophil infiltration and crypt abscesses, although anatomical distribution of the lesions went against UC (Chen et al. 2020a; Shimizu et al. 2020; Taniguchi et al. 2021; Zanatta et al. 2022; Zheng et al. 2018; Zou et al. 2020). In 33 patients with GI ulcers with or without colitis, endoscopic investigations could suggest Crohn's disease, although detailed data would be needed to properly classify patients. Histological analyses revealed inflammatory infiltrate of neutrophils, lymphocytes and eosinophils, as well as tissue granulation. Granuloma were described in 2 patients (Mitsunaga et al. 2022; Wu et al. 2021).

Perianal inflammation affected 27 patients (Aeschlimann et al. 2018; Franco-Jarava et al. 2018; Girardelli et al. 2021; Hori et al. 2019; Jiang et al. 2022; Jo et al. 2022; Kadowaki et al. 2018; Li et al. 2019; Liu et al. 2023; Rossi et al. 2022; Shimizu et al. 2020; Taniguchi et al. 2021; Tian et al. 2022; Tsuchida et al. 2019; Zhang et al. 2022; Zheng et al. 2018; Zhou et al. 2016; Zou et al. 2020). It included peri-anal ulcers, fistulae and abscesses. It was significantly more frequent in men (17 vs 10 patients, p=0.01). Fifteen of them displayed associated GI ulcers and could be classified as IBD.

Only 2 patients exhibited isolated GI involvements: 48 displayed joint (n=13), skin (n=18) or both (n=17) involvements, while 17 other had history of recurrent fever, and 10 other organ involvements (CNS, autoimmunity, immune deficiency). The systemic presentation of patients with GI involvement, as well as the young age of onset, may distinguish HA20 from other forms of IBD.

Liver involvement of HA20 has been reviewed by Deshayes *et al.* Since then and overall, it has been described in 17 patients (10%) (Cao et al. 2023; Deshayes et al. 2021; Duncan et al. 2018; Gans et al. 2020; He et al. 2020; Hori et al. 2019; Kim et al. 2020; Li et al. 2019; Rajamäki et al. 2018; Schwartz et al. 2020; Takagi et al. 2018; Takagi et al. 2017; Taniguchi et al. 2021; Yan et al. 2021; Zheng et al. 2018). The severity ranged from elevated liver enzymes to cryptogenic cirrhosis with liver failure. However, the prevalence of subclinical liver disease

may be underreported. When specified, the histologic analyses revealed interface hepatitis with lymphocyte infiltration and fibrosis.

D. Skin manifestations

Skin features were reported in 76 patients (43%). The most frequent manifestations were pseudo/folliculitis, pustules, acne-like lesions and dermal abscesses of the face and the trunk (29 patients, 16%). Seventeen patients (10%) displayed non-specific salmon or erythematous rash, with skin biopsies showing a neutrophilic or lymphocytic infiltrate without sign of vasculitis. Psoriasis, vitiligo, and urticaria were described in 4 patients each (Alhebshi et al. 2020; Berteau et al. 2019; Berteau et al. 2019; Harris et al. 2018; Kadowaki et al. 2018; Niwano et al. 2022; Rajamäki et al. 2018; Zanatta et al. 2022) (cold urticaria in 3 of the same family, and unspecified in 1). 2 patients had displayed local skin reactions after vaccination (Crustose after BCG vaccine and severe swelling after pneumococcal unconjugated vaccine) (Berteau et al. 2019; Hori et al. 2019). Malar rash and alopecia was described in 4 different patients, 3 of which were diagnosed with SLE (Aeschlimann et al. 2018; Shaheen et al. 2021; Zhang et al. 2022; Zhang et al. 2021). Panniculitis was described in 10 patients, including erythema nodosum in 8 (Chen et al. 2020a; He et al. 2020; Kadowaki et al. 2018; Sato et al. 2018; Tian et al. 2022; Tsuchida et al. 2019), necrotic panniculitis of the limbs in 1 (Zanatta et al. 2022) and generalized erythematous wheal-like patches in 1 patient (Kim et al. 2020). Seven patients exhibited a vasculitic rash, including 3 IgA vasculitis (Kadowaki et al. 2018; Viel et al. 2018; Zhang et al. 2022), 2 unspecified vasculitic rashes (Papadopoulou et al. 2019; Zhang et al. 2022), 1 purpura (Aeschlimann et al. 2018) and 1 vasculitis of the extremities (Chen et al. 2020a). 3 Patients displayed eczema (Aeschlimann et al. 2018; Wu et al. 2021) and 1 skin xerosis (He et al. 2020). Finally, other manifestations included geographic tongue (n=2) (Aeschlimann et al. 2018), pityriasis rosea (Aeschlimann et al. 2018), pernio-like lesion (Tsuchida et al. 2019), Raynaud phenomenon (Tsuchida et al. 2019), toenail ulcer (Liu et al. 2023), aquagenic acrokeratoderma (Ohnishi et al. 2017), neutrophilic dermatosis (Franco-Jarava et al. 2018) and Steven-Johnson syndrome (Aeschlimann et al. 2018)(n=1, each). Skin features were never isolated, and were associated with joint and/or GI symptoms in most cases (60 patients) or with autoimmunity, CNS or cardiovascular involvements in 14 other cases. Overall, 44 patients had history of fever and 55 had associated mouth ulcers, including 29 with genital ulcers.

E. Auto-immunity

Autoantibodies and/or autoimmune disease (AID) have been described in 60 patients (34%). The most frequently described were anti-thyroid autoantibodies (28 patients, 16%) and manifested themselves as an authentic Hashimoto thyroiditis in 24 patients (14%). Thyroiditis was never isolated: 19 patients displayed associated mouth ulcers. The 9 remaining patients exhibited associated GI involvements, genital ulcers and/or recurrent fever.

SLE had been diagnosed prior to HA20 diagnosis or as an associated disease in 16 patients, 13 of whom completed the SLICC criteria for SLE (Petri et al. 2012) (Aeschlimann et al. 2018; Duan et al. 2019; He et al. 2020; Kadowaki et al. 2021a; Kadowaki et al. 2018; Kim et al. 2020; Li et al. 2019; Miyamoto et al. 2022; Papadopoulou et al. 2019; Shaheen et al. 2021; Su et al. 2021; Zhang et al. 2022). Fourteen of them were women. Lupus nephritis was diagnosed in 7 patients (Li et al. 2019; Papadopoulou et al. 2019; Su et al. 2021; Zhang et al. 2022; Zhang et al. 2021). Median at first symptoms in patients diagnosed with SLE was 7.5 years old. Symptoms suggestive of SLE included arthritis/arthralgia (n=9), hemolytic anemia (n=4), lymphadenopathies (n=3), neuropsychiatric involvements (n=3), alopecia (n=2), malar rash (n=2) and Raynaud's phenomenon (n=1). However, history of recurrent fever was described in 9 patients, lung involvements in 3 and retinal vasculitis in 2. In 1 patient, diagnosis of SLE was questioned because of vertebral arthritis, sacroiliitis and pediatrics onset, leading to genetic analysis and HA20 diagnosis (Zhang et al. 2021).

Sixteen additional patients exhibited anti-nuclear antibodies, including five with anti-dsDNA antibodies and one with anti-extractable nuclear antigen antibodies (anti-RNP, anti-SSA/SSB, anti-scl70).

Immune cytopenia was described in 11 patients (6%), including autoimmune hemolytic anemia (9 patients)(Endo et al. 2022; Rossi et al. 2022; Su et al. 2021; Suri et al. 2021; Viel et al. 2018; Zhang et al. 2022; Zhang et al. 2021) and immune thrombocytopenia (4 patients)(Alhebshi et al. 2020; Endo et al. 2022; Viel et al. 2018; Wu et al. 2021). Immune cytopenia was associated with mouth/genital ulcers in 5 cases, fever in 3 cases, joint features and/or lymphadenopathies in 4 cases, and developmental delay in 3.

Five patients were diagnosed with 2 definite AIDs (Thyroiditis and type I diabetes n=2, Thyroiditis and haemolytic anemia n=1, SLE and thyroiditis n=1, SLE and type I diabetes n=1), and 7 families exhibited ≥2 AID in a single family.

F. Joint and musculoskeletal manifestations

Joint involvements were reported in 54 patients with HA20 (31%). Inflamed site was unspecified in 35 cases, affected large joints (wrists, ankles, knees, hip) in 12 cases (Berteau et al. 2019; El Khouri et al. 2023; Kim et al. 2020; Rossi et al. 2022; Tian et al. 2022; Tsuchida et al. 2019; Zhang et al. 2022; Zhang et al. 2022; Zou et al. 2020) and small joints in 4 (Hands, feet) (Aeschlimann et al. 2018; Berteau et al. 2019; Rossi et al. 2022; Tian et al. 2022). Sacroiliitis was described in 2 patients (Su et al. 2021; Zhang et al. 2021). Finally, 1 patient exhibited unspecified osteomyelitis while diagnosed with chronic granulomatous disease (CGD)(Suri et al. 2021). Among the patients with arthritis/arthralgia, none presented with rheumatoid-arthritis specific antibodies (rheumatoid factor, anti-CCP antibodies), except for a child with isolated sacroiliitis and rheumatoid factor (Su et al. 2021). Thirteen displayed anti-nuclear antibodies, and eight of them additionally displayed anti-dsDNA antibodies and anti-nuclear nuclear antigens (anti-ENA). Synovial fluid examination was not described. There has been no description of destructive arthritis or joint deformity. Of the 54 patients with involvement of the joints, 30 had GI involvement and 28 had skin features, which might distinguish HA20 from more common rheumatic diseases. Moreover, 40 patients displayed mouth ulcers, including 25 with genital ulcers.

G. Lymphadenopathy

Lymphadenopathies were described in 25 patients (14%) (Deshayes et al. 2021; Dong et al. 2019; Endo et al. 2022; Endo et al. 2020; Franco-Jarava et al. 2018; Hori et al. 2019; Jo et al. 2022; Kadowaki et al. 2021a; Kadowaki et al. 2018; Liu et al. 2023; Rossi et al. 2022; Shaheen et al. 2021; Sun et al. 2022; Takagi et al. 2017; Tian et al. 2022; Tsuchida et al. 2019; Zhang et al. 2022; Zou et al. 2020). They were located in the cervical area for 10 of them, the axillary and popliteal area in 1 each, and generalized in 11. Two patients had a probable diagnosis of autoimmune lymphoproliferative syndrome (ALPS) according to the 2010 diagnostic criteria (Endo et al. 2022; Oliveira et al. 2010; Takagi et al. 2017). They both exhibited chronic lymphadenopathies, elevated TCRαβ+ double-negative T cells and elevated serum FASL levels. Genetic testing for mutations in ALPS-associated genes was unconclusive in both

patients, including *FAS, FASL, KRAS, NRAS, PRKCD, PI3KCD, CTLA4*. (Endo et al. 2020; Takagi et al. 2017). Histological analyses of lymph nodes showed follicular hyperplasia with increased numbers of neutrophils and lymphocytes or necrotizing adenitis.

H. CNS manifestations

Seventeen patients (10%) displayed a wide spectrum of CNS manifestations, which causative relation to HA20 remains to be established.

Cerebral vessels were involved in four patients; 2 had small vessel vasculitis, and 2 had unspecified stroke during childhood, one of which additionally displayed central venous thrombosis (Aeschlimann et al. 2018; He et al. 2020).

Four patients had inflammatory CNS involvements, including two aseptic meningitis and another unspecified neuroinflammation (Hori et al. 2019; Schwartz et al. 2020; Shaheen et al. 2021). Finally, 1 patient exhibited intracerebral calcifications and aseptic necrotizing granulomatous mass lesions in the paracentral lobule and the thalamus with elevated expression of interferon-stimulated genes (ISG) suggesting interferonopathy (Mulhern et al. 2019).

Five additional patients exhibited developmental disorder without cause (developmental delay n=5, ADHD n=1, autism n=1) (Aeschlimann et al. 2018; Franco-Jarava et al. 2018; Su et al. 2021; Sun et al. 2022; Viel et al. 2018; Wu et al. 2021). Interestingly, 3 of the 5 patients with neurodevelopmental delay displayed large deletions of chromosome 6 (>5.5MB), therefore these manifestations may not be caused by HA20.

The other manifestations were included Epilepsy (n=2), craniopharyngioma (n=1), Type II Arnold-Chiari malformation (n=1), cerebral palsy due to neonatal cardiac arrest (n=1)(Duan et al. 2019; Hori et al. 2019; Kadowaki et al. 2021a; Suri et al. 2021).

I. Ophtalmologic manifestations

Ocular involvements of HA20 have recently been reviewed by Maccora et al (Maccora et al. 2021). Since then and overall, they affected 13 patients. Uveitis was described in 8 of them (anterior n=5, unspecified n=3) (Aeschlimann et al. 2018; El Khouri et al. 2023; Mulhern et al. 2019; Papadopoulou et al. 2019; Schwartz et al. 2020; Tian et al. 2022; Tsuchida et al. 2019) and three displayed retinal vasculitis (Aeschlimann et al. 2018; He et al. 2020). Finally, 2 had episcleritis and one had conjunctivitis, palpebral ulceration, and chorioretinitis, each (Berteau et al. 2019; El Khouri et al. 2023; Jiang et al. 2022; Mulhern et al. 2019; Ohnishi et al. 2017).

J. Lung manifestations

Ten patients (6%), including 8 women, had lung involvements. It consisted in interstitial lung disease in 5 patients, one of which during the course of hemophagocytic lymphohistiocytosis (HLH), and pulmonary nodules on CT imaging in 4 patients (Duncan et al. 2018; He et al. 2020; Hori et al. 2019; Li et al. 2019; Rajamäki et al. 2018; Yan et al. 2021; Zhang et al. 2022). One additional patient had bilateral pneumonia of unknown origin (Duan et al. 2019). Improvement after treatment was described in 6 patients. None progressed to lung fibrosis. Lung involvements were not isolated and were associated with mouth ulcers and/or autoimmunity in 6 patients each.

K. Cardiovascular manifestations

Cardiovascular manifestations were described in 14 patients (8%). Pericarditis was found in 5 patients, 3 of which were possibly not related to the immunological disease (infectious pericarditis, anasarca) (Aeschlimann et al. 2018; Deshayes et al. 2021; Li et al. 2019; Zhang et al. 2022). Vascular involvements were rare (5 patients, 3%). Veinous involvements were present in 4 patients and included pulmonary embolism caused by catheter-related veinous thrombosis, bilateral lower limbs thrombophlebitis, superficial thrombophlebitis and cerebral venous thrombosis (Aeschlimann et al. 2018; Berteau et al. 2019; He et al. 2020). All 4 patients displayed mouth ulcers and 3 of them (75%) qualified as Behçet's disorders according to the International Criteria for Behçet Disease (ICBD) (Disease (ITR-ICBD) et al. 2014). However, 3 had recurrent fever, 2 had GI and 2 lung involvements. Arterial involvements mostly affected the CNS (strokes n=2), although one patient was described with systemic polyarteritis nodosa-like vasculitis causing myocardial and kidney infarction as well as diffuse aneurisms (Niwano et al. 2022). Finally, 2 patients were described to have unspecified CNS vasculitis and 1 child to suffer from aortic valve insufficiency (Kadowaki et al. 2018).

L. Immunodeficiency

Nine patients (5%) were described to have definite immunodeficiency (Aeschlimann et al. 2018; Dong et al. 2019; Gans et al. 2020; Rajamäki et al. 2018; Shaheen et al. 2021; Suri et al. 2021). Recurrent bacterial and/or chronic viral infections were described in 5 of them, including

recurrent atypical pneumonia, ear and urinary tract infections, chronic EBV and papillomatosis. Immunological investigations revealed humoral deficiency in 5 patients (3%), Natural Killer cell (NK) deficiency in 3 patients (2%) including 2 patients with combined immune deficiency. Patients with humoral deficiency exhibited IgG subclass deficiency (n=5), low response to pneumococcal vaccine (n=4), and B cell lymphopenia (n=3). Isolated IgG deficiency with normal CD19 appeared in one patient after several years of treatment with hydroxychloroquine, MMF, prednisone and rituximab for SLE and could be secondary to treatments (Shaheen et al. 2021). Finally, unspecified CGD was described in one patient (Suri et al. 2021). Three patients required supplementation with intravenous immunoglobulin to reduce the number of infectious episodes.

M. Other

Ten patients from seven families had short stature (height < third percentiles) (He et al. 2020; Kim et al. 2020; Rossi et al. 2022; Zhang et al. 2022; Zhang et al. 2021). Six (60%) had never received corticosteroids before height evaluation. Two patients had evaluation of growth hormone that were normal. Therefore, short stature could be a consequence of prolonged systemic inflammation.

Two patients have been diagnosed with cancer; one patient with Hodgkin's lymphoma at the age of 21 years old, who later was diagnosed with craniopharyngioma at age 33, and the other one with diffuse large B-cell lymphoma at age 35 (Endo et al. 2022; Hori et al. 2019). Therefore, this review does not support the hypothesis of a higher risk of cancer in HA20 patients However, this result should be confirmed in cohort studies.

HLH was described in four patients. It was the first manifestation of HA20 in two of them, which occurred very early in life, at age 3 and 4 months (Aslani et al. 2022). In two other patients, HLH occurred 2 years after the onset of a systemic juvenile idiopathic arthritis (sJIA)-like disease (Li et al. 2019; Sun et al. 2022). Whole exome sequencing was performed in all patients, which revealed *TNFAIP3* mutations. The presence of mutations in HLH-related genes was not detailed.

Three patients from 3 different families have been reported to have menstrual cycle anomalies, including premature ovarian failure, late menarche and dysmenorrhea (Aeschlimann et al. 2018).

4/ Complications and mortality

Inflammatory (AA) amyloidosis has not yet been described as a complication of HA20. While it may be explained by the young age of the patients described, the absence of AA amyloidosis in other NF-κB-mediated autoinflammatory disease could suggest a low risk (Nigrovic et al. 2020).

Death was reported in 5 patients (3%). In 4 of them, death was thought to be a complication of HA20 and occurred before adulthood. One patient died of upper airway hemorrhage due to tonsillar ulcerations and carotid artery erosion at age 8 (Aeschlimann et al. 2018). One patient died of cerebral hemorrhage complicating HLH at age 4 (Aslani et al. 2022), one patient died of cytokine storm after hematopoietic stem cell transplant at age 15 (Wu et al. 2021), and one from of systemic inflammatory response syndrome (Sun et al. 2022). Finally, one adult patient with mild symptoms died of unknown cause (Dong et al. 2019).

5/ Genotype/phenotype correlation

By comparing patients based on the type of variations (missense variants (n=41 (23.2%) vs. ones leading to no or truncated form of A20 (pLOF), n=135 (76.3%)) we showed that pLOF variants were more associated to bipolar aphtosis, gastrointestinal involvement and autoimmunity (Supplementary Table 4, $p<0.005$). By splitting patients in two groups based on the localization of the protein affected by the mutations (OTU domain (n=92) vs. the other domains (n=85)), patients affected in the OTU domain had more genital aphtosis (47.4% in the OTU group vs. 20%, $p<0.002$) and skin involvement (53.3% in the OTU group vs. 31.8%, $p<0.01$) while the other had an enrichment in CNS involvement (16.5 % vs 4.3%, $p<0.02$) (Supplementary Table 5).

We then performed a multiple correspondence analysis (MCA) on clinical variables and the type of mutation (missense versus the other). By performing an unsupervised clustering (see methods), we ended-up with 3 clusters (Figure 2). The supplementary table 5 shows the repartition of the patients in each cluster with their clinical characteristics. Cluster 1 (n=108) was driven by the over-representation of autoimmunity (n=51, 48.1%, $p<0.001$) and missense variants ($p<0.02$). Cluster 2 (n=50) is driven by the presence of GI involvement (96%, $p<0.001$) while patients in this group had less bipolar aphtosis than in cluster 3. Finally, patients in the cluster 3 (n=19) had a high proportion of skin (89.5%) and joint involvement (84.2%) with almost all of them having bipolar aphtosis and a high proporition of GI involvement (Supplementary Table 6).

6/ Biological findings

Inflammatory markers usually rise during flares, including leukocyte count and C-reactive protein (CRP). CRP was elevated in 54 patients (87% of patients for which it was reported), with a median of 62mg/L. CRP remained elevated outside of flares in 7 out of the 19 patients for which it was specified. Hypergammaglobulinemia was observed in 13 patients with a median of 26.3 g/l [14.3-31.1].

Expectedly, serum pro-inflammatory cytokines, including IL-1β, IL-17, IL-8, IL-6 and TNFα, were elevated but were not systematically investigated.

Type I interferon signature was investigated in 21 patients from 6 different centers and was elevated in 17 of them. The score was reported in 9 patients. The median value was 880% of the respective laboratory normal values [382-1111]. The test was ordered before HA20 diagnosis in 3 patients; one with cerebral calcifications suggesting interferonopathy, one with colitis and panniculitis, and one with recurrent stomatitis. In 5 patients, it was ordered because of refractory HA20 with various manifestations including colitis, "neuroinflammation", membranous nephropathy and inflammatory liver disease. Finally, 8 patients were included in a study aiming to describe ISG in HA20 patients. The disease of the three patients with normal interferon signature was quiescent. Therefore, more data is needed to determine the predictive value of this test.

7/ Diagnosis

The diagnosis of HA20 relies in the detection of a germline variant in the gene *TNFAIP3* that is rare in the public databases and predicted to be deleterious *in silico*. More importantly previously unknown variants should be tested in vitro for their inhibition capacity towards NF-κB.

Previous diagnosis was described in 78 patients. Seventeen of them had received more than one diagnosis before HA20 diagnosis. Previous diagnoses included Behçet's disease (33 patients), Periodic fever, aphthous stomatitis, pharyngitis, and adenitis/PFAPA (9 patients), juvenile Idiopathic arthritis (8 patients), recurrent stomatitis (6 patients), Crohn's disease (6 patients), rheumatoid arthritis (5 patients) and connective tissue disease (3 patients). One patient was diagnosed with adult-onset still's disease, Kikuchi-Fujimoto disease, rheumatic fever,

Kawasaki disease, familial Mediterranean fever, IgA nephropathy and unclassified granulomatous neuroinflammatory disorder, respectively. Finally, 16 patients were diagnosed with systemic lupus erythematosus, although it can be considered as an associated diagnosis rather than a differential of HA20 (see autoimmunity).

8/ Treatments

To date, treatments in HA20 are not codified and are symptom oriented. The description of the treatments and their effectiveness was heterogeneous, and it was not possible to draw any clear conclusions. Treatments classified by predominant symptom and by therapeutic class are presented below.

GI manifestations were treated according to the principles of IBD treatment, with heterogenous responses (5-aminosalicylic acid n=9 (Berteau et al. 2019; He et al. 2020; Kadowaki et al. 2018; Li et al. 2019; Taniguchi et al. 2021; Tsuchida et al. 2019; Zheng et al. 2018), thalidomide n=13 (Chen et al. 2020a; He et al. 2020; Mitsunaga et al. 2022; Tian et al. 2022; Uchida et al. 2020; Zhang et al. 2022; Zheng et al. 2018), TNF blockers (n=17) (Duncan et al. 2018; Girardelli et al. 2021; He et al. 2020; Hori et al. 2019; Kadowaki et al. 2018; Li et al. 2019; Mitsunaga et al. 2022; Ohnishi et al. 2017; Shimizu et al. 2020; Uchida et al. 2020; Wu et al. 2021; Zanatta et al. 2022; Zhang et al. 2022; Zheng et al. 2018; Zou et al. 2020).

Joint manifestations were mainly treated by conventional and biologic disease-modifying anti-rheumatic drug (cDMARDS and bDMARDs) including methotrexate (n=12, effective in 3), azathioprine (n=4), sulfasalazine (n=3), ciclosporine (n=1) and TNF-α inhibitors (n=5) (Berteau et al. 2019; Deshayes et al. 2021; El Khouri et al. 2023; He et al. 2020; Lawless et al. 2018; Li et al. 2019; Ohnishi et al. 2017; Rossi et al. 2022; Shimizu et al. 2020; Su et al. 2021; Suri et al. 2021; Tian et al. 2022; Tsuchida et al. 2019; Uchida et al. 2020; Zhang et al. 2022; Zheng et al. 2018). Moreover, 3 patients received tofacitinib and 2 received tocilizumab with partial to good results (Schwartz et al. 2020; Mulhern et al. 2019; Ohnishi et al. 2017; Kadowaki et al. 2018).

Corticosteroids and NSAIDs were used in 32 patients and were useful in on-demand treatment of inflammatory attacks as well as alleviating auto-immune flares (Berteau et al. 2019; Duan et al. 2019; Duncan et al. 2018; El Khouri et al. 2023; Franco-Jarava et al. 2018; Girardelli et al. 2021; He et al. 2020; Kadowaki et al. 2021a; Kadowaki et al. 2018; Lawless et al. 2018; Li et

al. 2019; Mitsunaga et al. 2022; Mulhern et al. 2019; Ohnishi et al. 2017; Rossi et al. 2022; Su et al. 2021; Tian et al. 2022; Tsuchida et al. 2019; Yan et al. 2021; Zanatta et al. 2022; Zhang et al. 2022; Zheng et al. 2018).

Colchicine was used in 28 patients to relieve mucosal ulcers and inflammatory attacks, with good response described in 10 patients; partial and/or transient in 4 patients, and ineffective in 5 patients (Berteau et al. 2019; Debeljak et al. 2023; El Khouri et al. 2023; Hori et al. 2019; Horita et al. 2019; Jiang et al. 2022; Jo et al. 2022; Kadowaki et al. 2021a; Kadowaki et al. 2018; Lawless et al. 2018; Mitsunaga et al. 2022; Niwano et al. 2022; Shimizu et al. 2020; Suri et al. 2021; Taniguchi et al. 2021; Tsuchida et al. 2019; Uchida et al. 2020; Wakatsuki et al. 2023). Thalidomide was used in 5 patients for mouth ulcers with a partial or good response in 4 of them.

TNF-inhibitors were prescribed 29 patients, mainly for IBD (n=17), arthritis (n=5), liver involvement (n=3), lung involvement (n=2), bipolar ulcer, SLE and vasculitis (n=1, respectively). Efficacy was good in 11 patients, partial and/or transient in 3 patients, and ineffective in 2 patients. Tocilizumab have been used in 4 patients for joint inflammation (n=2), systemic inflammation and IBD (n=1, respectively) with partial to good response in 3 of them (Kadowaki et al. 2018; Lawless et al. 2018; Ohnishi et al. 2017; Uchida et al. 2020). Other treatments included mycophenolate mofetil (n=5) and methotrexate (n=14).

JAK inhibitors have been used with good efficacy in 6 patients to treat CNS involvements, uveitis and polyarthritis, all of which had high ISG prior to treatment (Aeschlimann et al. 2018; Mulhern et al. 2019; Schwartz et al. 2020).

Finally, four patients underwent hematopoietic stem cell transplantation (HSCT). Three had allogenic HSCT because of organ-damage uncontrolled with immunosuppressive agents. Complete chimerism was achieved in two patients with overall good outcome, despite the development of anti-GAD neuropathy in one (Shiraki et al. 2021b; Wu et al. 2021). On the contrary, one patient died of cytokine-mediated multi-organ injury a few hours after stem cell infusion (Wu et al. 2021). One patient had autologous HSCT for SLE-like CNS vasculitis, which allowed transient amelioration (Aeschlimann et al. 2018).

Discussion

In this systematic literature review we describe the clinical presentation of the 177 patients with HA20 from 94 families published. While the reported number of patients worldwide is small, HA20 is probably more frequent than reported. First, several family members described in paper were excluded because they had not undergone genetic testing while they had exhibited symptoms that could suggest HA20. Moreover, *TNFAIP3* sequencing may not be available worldwide. The diagnosis of sJIA or Behçet's disease in HA20 patients may not have been reconsidered, especially if the disease is controlled with standard therapy. Finally, we can suspect that many diagnosed patients with HA20 are not published because the variant is already known and/or the clinical picture is similar to what is already described. While patients were mainly originating from Asia and Europe, there is no evidence to suggest that HA20 may have a geographical predominance, which is to be confirmed with the democratization of sequencing techniques

The clinical spectrum of patients with HA20 is large and will probably broaden in the next few years with the generalization of *TNFAIP3* study. The core symptoms of HA20 include mouth, genital and gastrointestinal ulcerations, associated with unspecific joint manifestations and acne-like skin disease. However, most organs can be affected with lower frequency (CNS, lymph nodes, cardiovascular). Similarly, the severity of the disease ranges from asymptomatic to death-threatening organ-involvements.

This review summarizes the clinical picture of HA20 patients, which will allow to give better criteria to order genetic testing. Up to now, of the 75 variants referenced in the infevers database (https://infevers.umai-montpellier.fr/web/search.php?n=26), only 19 are validated to be pathogenic and the pathogenic classification remains to be validated for 56 variants. In this review, it is noteworthy that 5 patients had concomitant variants in other genes, and 7 had a genetic deletion comprising other genes than *TNFAIP3*. Therefore, the presence of clinical symptoms unusual to HA20 in patients with *TNFAIP3* variant could suggest either another variant in another gene or a complex mutation such as a large deletion of the chromosome 6. Indeed, to date, there are no validated criteria for the diagnosis of HA20. It is confirmed upon the discovery of a heterozygous loss-of-function mutation in the gene *TNFAIP3*. When the variant has not been previously classified as pathogenic (new private mutation, mutation rarely reported in a healthy population), functional analysis should be performed to confirm its pathogenicity, especially when the mutation is a missense mutation. One way to evaluate the mutated allele's ability to inhibit NF-κB activity is by ectopically expressing the mutant in a cell line capable of monitoring NF-κB activity under various stimuli. If the mutated allele does

not hinder NF-κB activity, similar to transfecting an empty vector plasmid, it can be classified as a loss-of-function allele. It is worth noting that assessing the capacity of patient cells to activate NF-κB through various techniques is important but may not definitively establish the mutation's role in TNFAIP3 in the observed phenotype. A combination of both ectopic expression and primary cell analysis is always preferable.

The familial segregation should fit with a dominantly inherited and highly penetrant disease. Of note, 4 subjects included in this review were asymptomatic and *TNFAIP3* variant was discovered upon the diagnosis of one of their relative, including 2 who displayed gonadal mosaicism. This result questions the diagnosis of patients with missense variants in *TNFAIP3* that are not classified as loss-of-function. Whether these variants act as a predisposing factor of an immune disease is an open question, but they may not drive the disease on their own. Further functional research could improve the robustness of these diagnoses.

The diagnosis of HA20 should be suspected upon the existence of mouth ulcers/stomatitis of dominant inheritance that started early in life, especially when associated with typical skin features, findings of IBD, or the occurrences of several AIDs in the family.

While symptoms usually begin in childhood, HA20 can also be suspected in late-onset AID. In this series, first symptoms occurred during adulthood in 7% of patients. Moreover, patients with "mild" symptoms such as mouth ulcers and acne may not report any symptoms because they have become so accustomed to it. In the end, unless there is familial history of HA20, the clinical features of HA20 may not be specific enough to order a specific sanger genetic testing of *TNFAIP3* but rather a NGS panel of AID comprising *TNFAIP3* or a whole-exome sequencing followed by a list-of-genes-supervised analysis. It is noteworthy that familial history may be lacking because of *de novo* mutations. However, genetic testing of both asymptomatic parents should be done as gonadal mosaicism is possible and could impact genetic counselling.

We looked for a genotype-phenotype correlation. Although most patients will present with oral aphtosis or fever that is not depending on the type of mutation (pLOF or missense for instance), pLOF variants were more associated to bipolar aphtosis, GI involvement and autoimmunity. This could suggest that the level of loss-of-inhibition of NF-κB by missense variants is less severe than pLOF ones. An unsupervised classification of patients in clusters was also performed to see if a pattern of characteristics may drive any groups. Interestingly, it ended with three clusters, one of them being overrepresented with autoimmunity and less bipolar aphtosis, the two others being different by the presence of skin and joint manifestations (mainly

in cluster 3) and the presence of a GI involvement (more represented in cluster 2). Although the percentage of variance in the dataset explained by this multiple correspondence analysis (MCA) is weak this analysis emphasizes the variability of clinical presentation in HA20. Beyond the scope of this work, it would be interesting to see if patients of these 3 clusters may respond differently to treatments. Importantly, it should be noted that the majority of the reported variants have not been tested in an ectopic expression system to precisely characterize whether they are hypomorphic or not. While variants leading to early stop codons are highly predicted to be deleterious, missense variants in *TNFAIP3* that have not been tested in vitro should be approached with caution before definitively classifying them as causing HA20. Our results differ from those of Chen et al, who had found a higher frequency of musculoskeletal disorders in patient with variants disrupting both OTU and ZNF domains, and no difference for genital ulcers, skin and CNS involvements. This discrepancy can be explained by the higher number of patients included in our series (88 vs 177 patients), and the inclusion of all patients with *TNFAIP3* variants, counting those with concomitant gene variant. As in other monogenic autoimmunity and autoinflammation, the wide variability of clinical expression in HA20 is challenging, including within the same variant or even in the same family. This strongly suggests that additional factors are at play in the onset of the clinical phenotype that are not elucidated yet, such as microbial triggers, microbiota modifications, second somatic events, and epigenetic modifications.

In 2021, Kadowaki compared the clinical presentation of patients from inside and outside East Asia. They had found that patients from East Asia displayed more recurrent fever and less autoimmune diseases than patients from outside of East Asia, which is confirmed by our work (Kadowaki et al. 2021b). Several additional clinical items differed between geographical regions, including thyroiditis, skin features and genital ulcers although type of mutations did not differ between groups. These analyses are limited by the incompleteness and/or ambiguity of ethnic descriptions in the source articles, which resulted in the exclusion of 81 patients.

Men and women may exhibit different clinical symptoms of HA20. Unlike in Behçet's disease, women are significantly more at risk to develop genital ulcers (Cansu et al. 2016). On the other hand, men are more likely to suffer from both perineal inflammation and gastrointestinal tract ulcers. It is important to note that there are twice as many women described with HA20 than men. Several factors may explain this finding. First, men may have milder diseases for which they do not seek medical advice. Indeed, women exhibit twice as much genital ulcers and their painfulness could urge to consult. Moreover, the occurrence of genital ulcers may lead to further

investigations as differential diagnoses are limited (Mauskar et al. 2020). On the contrary, males harbor more perianal inflammation, which is a frequent conditions in the general population, therefore raising less suspicion of underlying systemic disease (Sahnan et al. 2017).

This work has several limitations intrinsic to its retrospective nature. First, the richness of clinical data were highly heterogenous between studies. Little is known on variant penetrance in HA20. Moreover, as previously mentioned, variants' pathogenicity was not established in 76.8% of cases. Therefore, we have chosen to include all patients with a variant in *TNFAIP3* gene regardless of their symptoms, including asymptomatic patients. Future work should investigate the significance of untested variants to increase the phenotypic accuracy of HA20. Finally, multiple patients/families were reported in several different papers. However, efforts were made to include all available data for each patient and to ensure that no patient was included twice.

Elements of pathogenesis need to be clarified as HA20 presentation may drastically differ between patients. While we have provided insight on a genotype/phenotype correlation in HA20, the understanding of the specific involved immune pathways is needed to guide therapeutic decisions. While strong systemic inflammation could suggest the use of cytokine blockers (Lawless et al. 2018; Ohnishi et al. 2017), the presence of a high ISG could suggest the use of JAK inhibitors (Miyamoto et al. 2022; Mulhern et al. 2019; Schwartz et al. 2020). Patients with HA20 had received several lines of treatments that were not necessarily described in the papers and therefore the range and efficacy of these treatments requires further descriptions, as clinical trials are unlikely to take place in a short future.

Methods

A systematic review of clinical studies published in the medical literature was conducted to retrieve case reports and case series reporting patients with TNFAIP3 mutation. This study was not reviewed by an ethics committee, as all data used in this study were taken from previously published articles.

Search strategy and selection process

MEDLINE was searched through PUBMED and EMBASE using the PRISMA guidelines (6) from the first description of the disease in 2016 until August 2023, with no language restriction or publication date limit. The search strategy included the following search terms: ("A20 haploinsufficiency") and ("TNFAIP3"). All articles describing patients with a mutation of TNFAIP3 and their clinical symptoms were included. Untested obligate carriers were not included. Only full-text articles in English were included. Information was extracted on characteristics of trial participants, including sex, ethnicity and/or geographic origin, family history, age at disease onset and diagnosis, mutational status, clinical and biological characteristics. Ethnicity and geographical origin were extracted when mentioned in the original article. When unspecified, patients were classified according to country of publication. Geographical origin was classified as America, Europe, East Asia, West Asia and South Asia.

Articles were checked to detect patients described in duplicates to ensure all information published on a patient was considered. We identified for inclusion in this review 68 articles (Aeschlimann et al. 2018; Alhebshi et al. 2020; Aslani et al. 2022; Berteau et al. 2019; Cao et al. 2023; Chen et al. 2020a; Debeljak et al. 2023; Deshayes et al. 2021; Dong et al. 2019; Duan et al. 2019; Duncan et al. 2018; El Khouri et al. 2023; Endo et al. 2022; Endo et al. 2020; Franco-Jarava et al. 2018; Gans et al. 2020; Girardelli et al. 2021; Harris et al. 2018; Hautala et al. 2020; He et al. 2020; Hori et al. 2019; Horita et al. 2019; Imai et al. 2020; Jiang et al. 2022; Jo et al. 2022; Kadowaki et al. 2021a; Kadowaki et al. 2018; Kim et al. 2020; Lawless et al. 2018; Li et al. 2019; Liang et al. 2019; Liu et al. 2023; Mitsunaga et al. 2022; Miyamoto et al. 2022; Mulhern et al. 2019; Niwano et al. 2022; Ohnishi et al. 2017; Papadopoulou et al. 2019; Rajamäki et al. 2018; Rossi et al. 2022; Sato et al. 2018; Schwartz et al. 2020; Shaheen et al. 2021; Shigemura et al. 2016; Shimizu et al. 2020; Shiraki et al. 2021b; Shiraki et al. 2021a; Su et al. 2021; Sun et al. 2022; Suri et al. 2021; Suzuki et al. 2017; Takagi et al. 2018; Takagi et al. 2017; Taniguchi et al. 2021; Tian et al. 2022; Tsuchida et al. 2019; Uchida et al. 2020; Viel et al. 2018; Wakatsuki et al. 2023; Wu et al. 2021; Yan et al. 2021; Ye et al. 2017; Zanatta et

al. 2022; Zhang et al. 2022; Zhang et al. 2021; Zheng et al. 2018; Zhou et al. 2016; Zou et al. 2020) describing 177 patients (supplementary figure 1). Patients are detailed in supplementary table 1)

Statistical analysis

The categorical variables were analyzed by using the chi-square test or Fisher's exact probability test as appropriate. Continuous variables in two or three groups were compared using the Mann-Whitney test or the Kruskal-Wallis test, respectively. The significance level was set a $p<0.05$ for all analyzed data. Statistical analysis was performed with the online application EasyMedStat (version 3.21.5 ; www.easymedstat.com). Clustering analysis are described in supplementary files.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Conflict of interest

The Authors declare no conflict of interest concerning this study.

Author contributions

Conceptualization: IE, SGL
Methodology: IE, SGL
Data Curation, IE, QR
Formal analysis: IE, QR
Writing - Original Draft: IE, QR
Writing - Review & Editing: IE, QR, GB, VH, FRL, SGL
Supervision: SGL

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Table 1. Main features of the 177 HA20 patients.

	n=177 (%) or median [range]
Women	109 (62)
Age at publication	15 [1-71]
Age at onset (years)	4 [0-35]
Diagnostic delay (years)	7 [0-55]
Dominantly inherited mutation	93/112 (82)
General	
Fever	93 (54)
Lymphadenopathy	25 (14)
Mucosal inflammation	129 (73)
Mouth ulcers	123 (69)
Genital ulcers	63 (36)
Gastrointestinal involvement	81 (46)
Abdominal pain	48 (28)
Diarrhea	41 (23)
Bloody stool	21 (12)
Gastrointestinal tract ulcer	43 (25)
Perianal inflammation	27 (15)
Liver involvements	17 (10)
Skin features	76 (43)
Pseudofolliculitis/Acne/Pustulosis	29 (16)
Rash	17 (10)
Panniculitis	10 (6)
Auto-immunity	60 (34)
Thyroïditis	24 (14)
Lupus	16 (9)
Immune cytopenia	11 (6)
Type I diabetes	6 (3)
Arthralgia/Arthritis	54 (31)
Central nervous system involvement	17 (10)
Cardiovascular involvement	14 (8)
Pericardial effusion	5 (3)
Venous thromboembolism	4 (2)
Vasculitis	3 (2)

Eye involvement		13 (8)
	Anterior uveitis	8 (5)
	Retinal vasculitis	3 (2)
Lung involvement		10 (6)
Immune deficiency		9 (5)
Elevated CRP during flares		54/63 (86)
C-reactive protein (mg/L) in flare		51 [0-450]

1

2

Figure legends

Figure 1. **Characteristics of HA20 patients described.**

(a) Schematic representation of reported mutations in *TNFAIP3* gene. (b) Geographic distribution (c) Main clinical features.

figure 2. **Hierarchical clustering of HA20 patients based on their characteristics (multiple correspondence analysis, MCA).**

Figure 2: Hierarchical clustering of HA20 patients based on their characteristics (multiple correspondance analysis, MCA)

(a) Hierarchical tree (dendogram) indicating how patients (x-axis, n=159) are clusterd in three groups (vertical lines). From left to right : cluster 1, 2 and 3.

(b) Heatmap showing the frequency of each characteristic among each cluster

(c) Factorial map where each dot represents one patient (n=159), colored based on the cluster they belong to (MCA analysis)

Table legends

Table 1. **Demographics and clinical features of the 177 HA20 patients included**

(a) Map of described mutations of TNFAIP3. (b) Geographical distribution of HA20 patients reported. (c) Representation of most frequent involvements in HA20.

Supplementary files.

Supplementary methods. **clustering analyses**

Supplementary figure 1. **Prisma flowchart.**

We identified for inclusion in this review 68 articles describing 177 patients. The search of Medline database provided 4025 articles. After adjusting for duplicates, 1588 remained. Of these, 1474 were discarded after reviewing the title because they were irrelevant. Forty-two citations were excluded because they were reviews, letter to the editor or replies. The full texts of the remaining 72 citations were examined in more details. Four reports were excluded because the clinical data were not detailed. Patients described in duplicate (n=36) and triplicate (n=4) were included once. Finally, 177 patients from 66 reports were included.

Supplementary Table 1. Detailed characteristics of the 177 patients included

Supplementary Table 2. Type of variation and domain affected in HA20 patients

Supplementary Table 3. Clinical features according to reported ethnicity and sex

Supplementary Table 4. Clinical features according to type of variation in *TNFAIP3* (pLOF vs Missense)

Supplementary Table 5. Clinical features according to *A20* domain affected (OTU vs non-OTU)

Supplementary Table 6. Clinical features according to clusters.

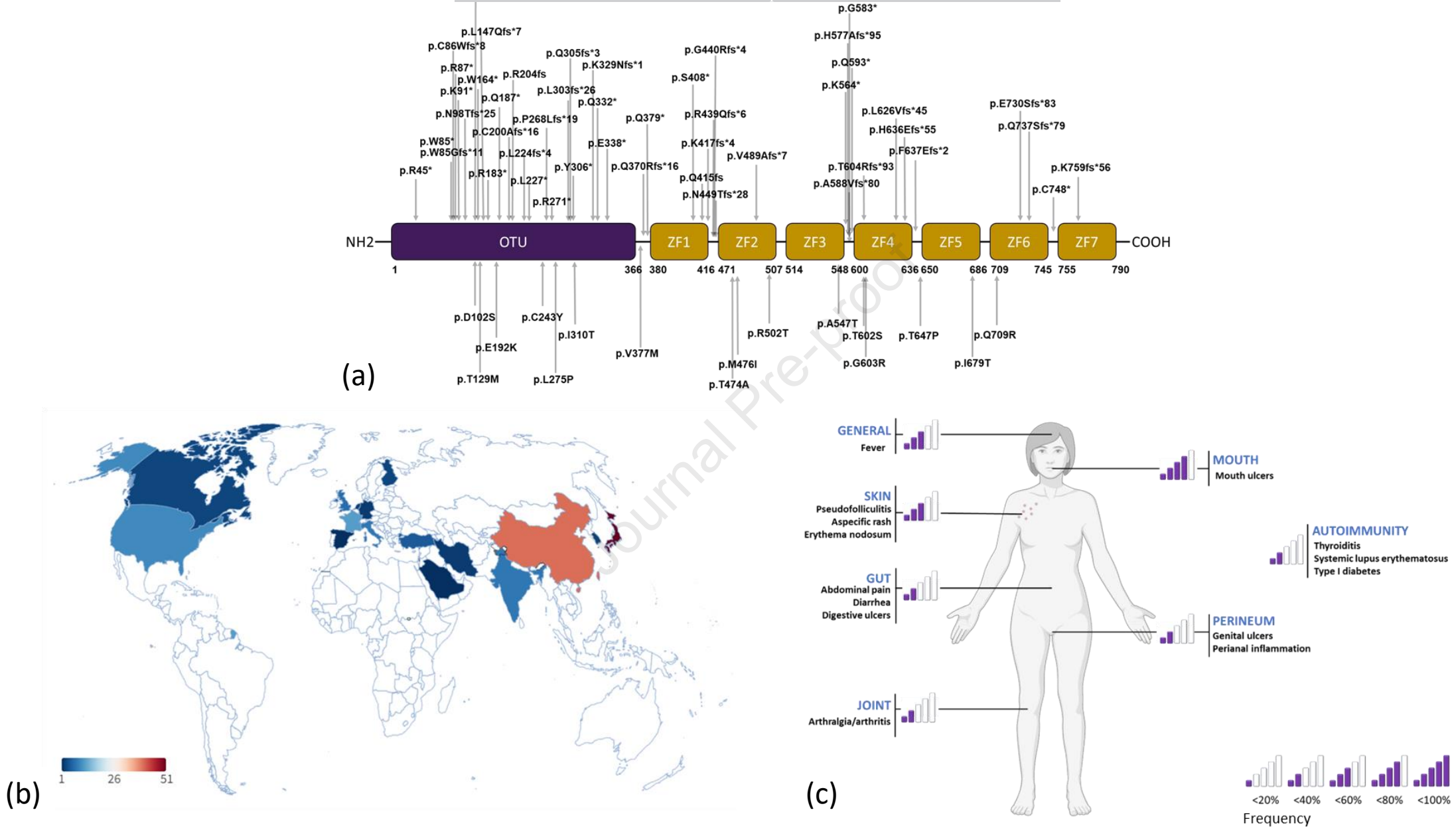

Figure 1. Characteristics of included patients with A20 haploinsufficiency (HA20)
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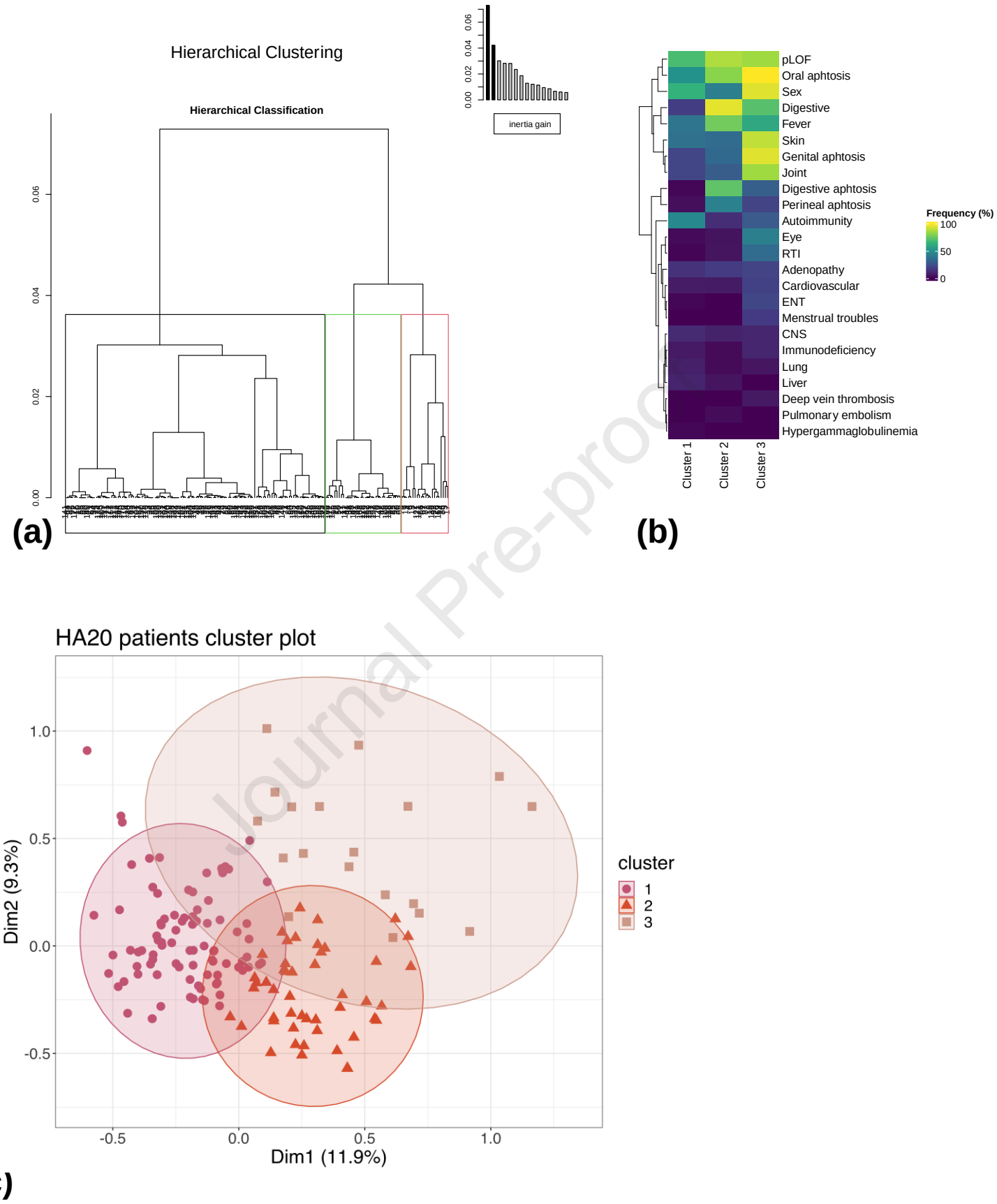

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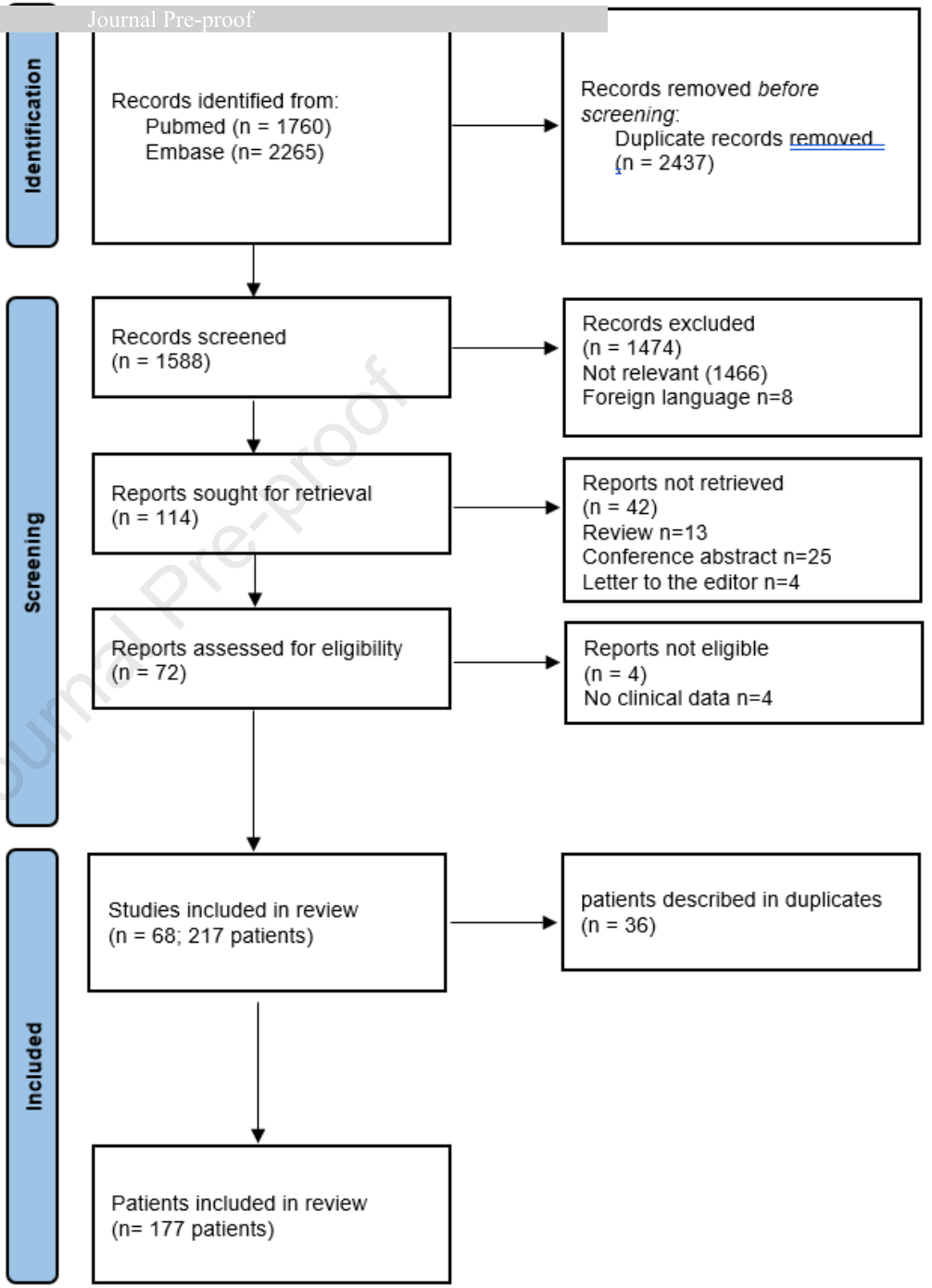

Supp. figure 1. Prisma flowchart
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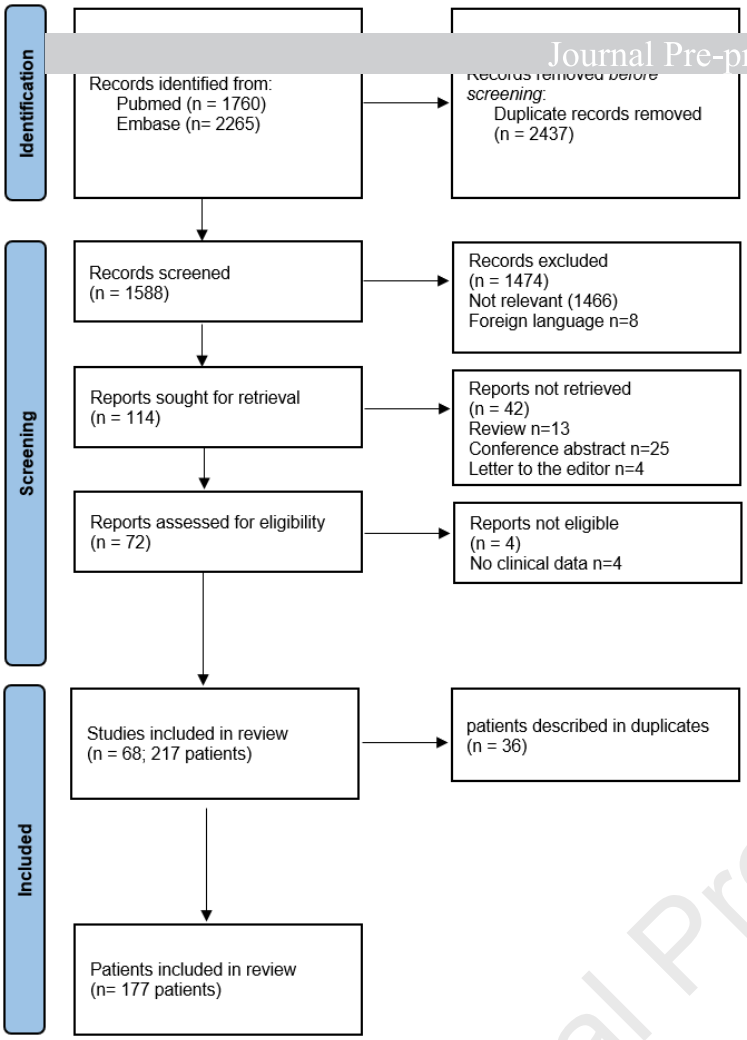

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Supplementary Table 3. Clinical features according to reported Georgaphic origin and sex

	East Asia n=103	Europe n=39	America n=16	West Asia n=10	South Asia n=8	p-value		Female n=108	Male n=69	p-value
Women	60 (58)	25 (64)	13 (81)	5 (50)	5 (63)	0.43				
Fever	62 (62)	14 (33)	9 (64)	5 (50)	2 (29)	**0.036**		54 (53)	38 (55)	0.7
Genital ulcers	30 (29)	18 (46)	8 (50)	6 (60)	1 (13)	**0.047**		48 (44)	15 (22)	**0.004**
Perianal inflammation	20 (19)	3 (8)	3 (19)	0	0	0.2		10 (10)	17 (27)	**0.01**
Digestive tract ulcer	32 (31)	5 (13)	2 (13)	1 (10)	2 (25)	0.1		19 (18)	24 (35)	**0.019**
Skin features	35 (34)	23 (59)	12 (75)	3 (30)	2 (25)	**0.003**		53 (50)	23 (32)	0.056
Anterior uveitis	2 (2)	2 (5)	3 (12)	0	1 (13)	**0.03**		7 (6)	1 (2)	0.1
SNC involvement	5 (5)	3 (8)	4 (25)	2 (20)	2 (25)	**0.015**		12 (12)	5 (7)	0.45
Autoimmunity	26 (26)	20 (51)	7 (44)	6 (10)	1 (13)	**0.01**		41 (39)	20 (26)	0.28
Thyroiditis	8 (8)	12 (31)	1 (6)	3 (30)	0	**0.003**		19 (18)	5 (7)	0.07

Supplementary methods. clustering analyses

Clustering analysis was performed in R version 4.0.3 using the packages *FactoMineR*, *factoextra*, *ClusterR*, *cluster* and *missMDA* and visualization using ggplot2. Briefly, only categorical variables were kept in this analysis. Missing data were imputed by using the function imputeFAMD function from the *missMDA* package with the method "regularized". The (regularized) iterative FAMD algorithm first consists in coding the categorical variables using the indicator matrix of dummy variables. Then, in the initialization step, missing values are imputed with initial values such as the mean of the variable for the continuous variables and the proportion of the category for each category using the non-missing entries. Multiple correspondence analysis on categorical variables was performed with the MCA function without any modification in the function. Patients were clusterized based on this result with the function HCPC which performs an agglomerative hierarchical clustering on results from a factor analysis with "ward" method. The number of clusters was set to -1 meaning that the number was unsupervised. Visualization of results and analysis of differences between clusters was performed by using *TableOne*, *ggplot2,* and *ggrepel* packages. All methods used here is available on this website: https://rpubs.com/nchelaru/famd.

Supplementary Tables

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