

## EXTENDED REPORT

# A20 haploinsufficiency (HA20): clinical phenotypes and disease course of patients with a newly recognised NF- $\kappa$ B-mediated autoinflammatory disease

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**Handling editor** Josef S Smolen

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/annrheumdis-2017-212403>).

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Received 18 September 2017  
Revised 15 December 2017  
Accepted 23 December 2017  
Published Online First  
9 January 2018

## ABSTRACT

**Objectives** The association between mutations in *TNFAIP3*, encoding the NF- $\kappa$ B regulatory protein A20, and a new autoinflammatory disease has recently been recognised. This study aims at describing the clinical phenotypes and disease course of patients with A20 haploinsufficiency (HA20).

**Methods** Data for all cases from the initial publication, and additional cases identified through collaborations since, were collected using standardised data collection forms.

**Results** A total of 16 patients (13 female) from seven families with a genetic diagnosis of HA20 were included. The disease commonly manifested in early childhood (range: first week of life to 29 years of age). The main clinical symptoms were recurrent oral, genital and/or gastrointestinal ulcers (16/16), musculoskeletal (9/16) and gastrointestinal complaints (9/16), cutaneous lesions (8/16), episodic fever (7/16), and recurrent infections (7/16). Clinical phenotypes varied considerably, even within families. Relapsing-remitting disease course was most common, and one patient died. Laboratory abnormalities included elevated acute-phase reactants and fluctuating presence of various autoantibodies such as antinuclear antibodies (4/10 patients tested) and anti-dsDNA (2/5). Tissue biopsy of different sites revealed non-specific chronic inflammation (6/12 patients tested), findings consistent with class V lupus nephritis in one patient, and pustules and normal results in two patients each. All patients were treated: 4/16 received colchicine and 12/16 various immunosuppressive agents. Cytokine inhibitors effectively suppressed systemic inflammation in 7/9 patients.

**Conclusions** Early-onset recurrent oral, genital and/or gastrointestinal ulcers are the hallmark feature of HA20. Frequency and intensity of other clinical manifestations varied highly. Treatment regimens should be based on disease severity, and cytokine inhibitors are often required to control relapses.

## INTRODUCTION

The protein A20, also known as TNAP3, encoded by *TNFAIP3*, plays a crucial role in the negative regulation of inflammation and immunity.<sup>1</sup> *TNFAIP3* is an ubiquitin-editing (deubiquitinase; DUB) enzyme

with a critical function in the inhibition of key proinflammatory molecules, including inhibitor of nuclear factor kappa B kinase subunit gamma (IKK $\gamma$  (NEMO)) and receptor-interacting protein kinase 1 (RIPK1), in the canonical NF- $\kappa$ B and other signalling pathways.<sup>2</sup> Zhou and colleagues<sup>3</sup> recently described a new autoinflammatory disease caused by heterozygous loss-of-function mutations in *TNFAIP3*, leading to haploinsufficiency of A20 (HA20). These mutations cause insufficient DUB activity of A20 and lead to increased NF- $\kappa$ B signalling and phosphorylation of c-Jun N-terminal kinase and p38 mitogen-activated protein kinases (MAPKs). HA20-associated mutations were found in six unrelated families who presented with mostly childhood-onset systemic inflammation and a ‘Behçet-like’ disorder that may lead to end-organ damage and death. Since the initial description, a few additional cases of HA20 have been reported in the literature.<sup>4–7</sup> To date, the clinical manifestations, severity of symptoms, disease course and complications of this newly described disorder are not well described and appreciated. Early recognition and diagnosis are crucial, as targeted therapies may alter disease course and improve outcome.

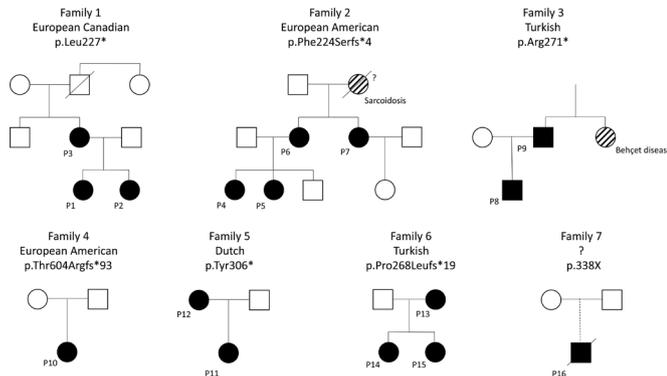
Therefore, the aims of the study were (1) to describe the disease features and course, treatment regimens, complications and outcomes of patients; and (2) to improve clinical recognition of this poorly defined disorder.

## PATIENTS AND METHODS

Sixteen patients from six unrelated families previously identified and diagnosed with HA20 by Zhou and colleagues<sup>3</sup> at the National Institutes of Health in the USA were initially included in the study. The patients were followed at various centres worldwide and their charts were retrospectively reviewed by their primary care physicians. Because the spectrum of clinical phenotypes associated with HA20 is still widely unknown, these patients’ histories were used to prepare a comprehensive data collection form including all signs and symptoms thought to be related to HA20. In the second step, patient charts were again reviewed by the primary care physicians using the newly created



**To cite:** Aeschlimann FA, Batu ED, Canna SW, et al. *Ann Rheum Dis* 2018;**77**:728–735.



**Figure 1** Pedigrees of the seven families with mutations in *TNFAIP3* leading to haploinsufficiency of A20 (HA20). The grandmother of family 2 was diagnosed with sarcoidosis and is thought to be affected by HA20, however died years prior to availability of genotyping. Patient 16 was adopted, and the ethnic origin is unknown.

data collection forms in order to collect detailed information. Demographic, clinical, laboratory, imaging and histological features were recorded. Disease course, treatment regimens, and disease- and treatment-related complications were captured. Additional cases were sought and one identified subsequently through collaborations.

All data were compiled and analysed using descriptive statistics. Patient consent, or consent from a parent in the case of children, was obtained by the responsible physician in the respective institutions.

**RESULTS**

A total of 16 patients (81% female) from seven families were included (figure 1 and table 1). All patients were Caucasian, while the ethnic origin of patient 16 is unknown (figure 1).

No consanguinity was reported within the families. Prior to the recognition of HA20, patients were diagnosed with various conditions including Behçet disease (BD), systemic lupus erythematosus (SLE), juvenile idiopathic arthritis (JIA), periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA), and inflammatory bowel disease (IBD). The grandmother of family 2 was diagnosed with sarcoidosis and succumbed to the complications of the disease. Given her medical history she is presumed to carry the F224Sfs\*4 mutation present in four affected family members; however, her DNA sample was not available for genotyping. Thus, the molecular diagnosis of HA20 has not been confirmed and this patient was not included in this analysis.

The demographic features of the patients are summarised in table 1.

**Disease presentation**

All patients were symptomatic and reduced penetrance was not reported in any family members. First symptoms occurred early, before 10 years of age in 11/16 (69%) patients, and disease onset ranged from the first week of life to 29 years of age. Clinical presentation was heterogeneous between families and between family members with the same mutation (figure 1 and table 2). In 10/16 (63%) patients, recurrent oral and/or genital ulcers were the symptoms leading to initial specialised medical consultation.

**Disease course**

During disease course, symptoms and severity were highly variable. Clinical features emerged over a period of several years. All patients developed recurrent painful oral, genital and/or gastrointestinal ulcers. Other common symptoms that occurred at various time points during disease course included gastrointestinal complaints (9/16, 56%), polyarthritis and/or arthralgia (9/16, 56%), skin involvement (8/16, 50%) and recurrent fever

**Table 1** Characteristics of patients with A20 haploinsufficiency

Patient no	Family	Sex	Current age	Age at onset	Previous diagnosis	Previous treatment	Current treatment
1	Family 1	F	25 years	10 months	JIA, Behçet disease	CS, MTX, CYS	MTX, thalidomide
2	Family 1	F	23 years	15 months	JIA, Behçet disease	CS, MTX, CYS, AZA, thalidomide, IVIG, ETN	IFX
3	Family 1	F	51 years	Early 20s	Rheumatoid arthritis (RF-), Behçet disease	None	Colchicine
4	Family 2	F	25 years	10 years	JIA, undifferentiated connective tissue disease/cutaneous vasculitis, SLE with CNS vasculitis	CS, MMF, ETN, thalidomide, RTX, IFX, MTX, ADA, cyclo, IVIG, autologous haematopoietic stem cell transplant	Anakinra, AZA, CS
5	Family 2	F	29 years	Around 8 years	Behçet disease, lupus nephritis	CS, colchicine, ETN, hydroxychloroquine	Anakinra
6	Family 2	F	51 years	Around 6 years	Rheumatic fever, arthritis	None	Anakinra
7	Family 2	F	56 years	Around 6 years		None	Anakinra
8	Family 3	M	9 years	9 months	Behçet disease	CS, colchicine, AZA	Colchicine
9	Family 3	M	46 years	?	Behçet disease		On treatment (not specified)
10	Family 4	F	15 years	8 weeks	Suspicion of PFAPA	CS, colchicine, ETN, anakinra, ADA, MTX	Tofacitinib
11	Family 5	F	17 years	Around 4 years	Suspicion of Behçet disease	CYS, dapsone, CS, AZA, IVIG for Ig deficiency	IFX
12	Family 5	F	47 years	Infancy	Suspicion of Behçet disease	CS, colchicine, AZA, IFX, IVIG for Ig deficiency	None
13	Family 6	F	38 years	29 years	Behçet disease	None	Colchicine
14	Family 6	F	19 years	15 years	Suspicion of Behçet disease	None	Colchicine
15	Family 6	F	15 years	13 years	Suspicion of Behçet disease	None	Colchicine
16	Family 7	M	(8 years)†	1 week	Crohn's disease, Behçet disease	CS, mesalamine, dapsone, MTX, AZA, colchicine, IFX, ADA, certolizumab, anakinra, canakinumab, tacrolimus, IVIG, tocilizumab	NA

ADA, adalimumab; AZA, azathioprine; CNS, central nervous system; CS, systemic corticosteroids; cyclo, cyclophosphamide; CYS, ciclosporin; ETN, etanercept; F, female; IFX, infliximab; IVIG, intravenous immunoglobulin; JIA, juvenile idiopathic arthritis; M, male; MTX, methotrexate; NA, not applicable; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, adenitis; RF, rheumatoid factor; SLE, systemic lupus erythematosus.

**Table 2** Clinical features of the patients with HA20 haploinsufficiency

Patient no	Fever	Ulcers	Musculoskeletal	Ocular	Skin	Cardiovascular	Intestinal	Other	Disease-related or treatment-related complications	Other symptoms possibly related to HA20
1	With flares	Oral, genital and perineal	Oligoarthritis, then polyarthritis*	Bilateral anterior uveitis	None	None	Bloody diarrhoea	Recurrent URTI and tonsillitis during childhood resulting in tonsillectomy; sometimes LAD (axillary, inguinal) with flares	Severe Cushing syndrome growth retardation, cataract and glaucoma requiring multiple ocular surgeries	Immature ovarian failure, geographic tongue, dental crowding secondary to small jaw
2	None	Oral, genital and perineal	Polyarthritis with dactylitis*	Bilateral anterior uveitis	None	None	Bloody diarrhoea		Cushing syndrome, cataract and glaucoma requiring multiple ocular surgeries	Acne during adolescence, eczema, pityriasis rosea, geographic tongue, dental crowding secondary to small jaw
3	None	Oral and genital*	Polyarthritis	None	Painful pustules in the genital region, breasts, axillae and sacrum, dermal abscess	None	None	Recurrent RTI during childhood		Acne during adolescence, thyroid goitre treated with radiation
4	Intermittent	Oral, genital and intestinal	Polyarthritis*	Retinal vasculitis, anterior uveitis	Stevens-Johnson syndrome, malar rash, painful subcutaneous rash, purpura, pustular, folliculitis-like rash on the extremities	None	Diarrhoea, secondary malabsorption syndrome	CNS vasculitis, idiopathic thrombocytopenic purpura	Steroid-induced diabetes mellitus	
5	None	Oral and genital*	Polyarthritis	None	Pustular, folliculitis-like rash on the extremities	None	None	Suspicion of pre-eclampsia during pregnancies due to arterial hypertension and proteinuria, later diagnosis of membranous nephropathy		Irregular menses, late menarche (at age 18 years)
6	Intermittent	Oral and genital*	Polyarthritis	None	Folliculitis, palmoplantar pustules	None	Diarrhoea, once bloody	Recurrent URTI and tonsillitis during childhood, occasional LAD (neck), intermittent arterial hypertension		Eczema, periodontal disease since childhood, recurrent unintentional weight loss
7	With flares	Oral and genital*	Polyarthritis	None	Folliculitis	None	None	Recurrent RTI resulting in tonsillectomy	Removal of fibroids complicated by severe staphylococcus infection	Asthma, enamel loss, uterine fibroids, status postgastric bypass for obesity
8	With flares	Oral, genital and intestinal	Arthralgia	None	None	Pericardial effusion*	Bloody diarrhoea		Recurrent URTI under azathioprine according to parents	
9	Recurrent*	Oral and genital*	None	None	None	None	None			
10	Recurrent*	Oral, rectal and perineal	None	None	None	Pericardial effusion	Abdominal pain, anorexia, nausea, vomiting, weight loss	Recurrent RTI during childhood (starting at 8 weeks of age) resulting in adenoidectomy and tonsillectomy, cervical LAD with fevers		Scoliosis requiring brace and subsequent surgical fusion therapy, vesicoureteral reflux surgery (pyelonephritis), attention deficit hyperactivity disorder
11	None	Oral and genital*	None	None	None	None	Abdominal pain, weight loss	Recurrent URTI and otitis media during childhood, requiring repeated tympanostomy, recurrent urinary tract infections		Cerebral palsy due to perinatal lacunar infarction, autism, severe foot pain associated with neurological monoplegia due to cerebral palsy requiring gabapentin and amitriptyline, recurrent headaches

Continued

Table 2 Continued

Patient no	Fever	Ulcers	Musculoskeletal	Ocular	Skin	Cardiovascular	Intestinal	Other	Disease-related or treatment-related complications	Other symptoms possibly related to HA20
12	None	Oral and genital*	Arthralgia	None	Severe acne in adulthood in the face, inguinal region and buttocks	None	Abdominal pain, diarrhoea, rectal bleeding	Recurrent RTI especially during childhood, asthmatic bronchitis	Bilateral pneumonia complicated by empyema, drainage and 3x streptokinase treatment and debridement (fight lung) with debridement (pus evacuation), azathioprine-induced severe lymphopaenia and neutropenia	Dysmenorrhoea resulting in hysterectomy, rosacea
13	With flares (low-grade)	Oral and genital*	Myalgia	None	Acne-like rash on the extremities	None	None	None	Hypothyroidism, arthralgia and myalgia (diagnosis of fibromyalgia), 'Dental problems'	
14	None	Oral and genital*	None	None	None	None	None	None		
15	None	Oral and genital*	None	None	None	None	None	None		
16	With flares*	Oral, intestinal and perineal	None	None	Non-specific rash	Pulmonary embolism	Poor feeding, abdominal pain* bloody diarrhoea, bowel perforation	Small vessel CNS vasculitis, inflammatory fibroepithelial polyp on the arytoid	Posterior reversible encephalopathy syndrome, steroid-induced vertebral compression fractures, cataracts, hyperglycaemia and arterial hypertension	

\*First symptom/finding leading to medical consultation.

CNS, central nervous system; HA20, A20 haploinsufficiency; LAD, lymphadenopathy; RTI, respiratory tract infection; URTI, upper respiratory tract infection.

Table 3 Overview of clinical and laboratory characteristics observed in patients with A20 haploinsufficiency

Features	Patients, n (%)
Mucous membrane	16 (100)
Musculoskeletal	9 (56)
Arthritis	7 (44)
Gastrointestinal	9 (56)
(Bloody) diarrhoea	5 (31)
Recurrent fever	8 (50)
Cutaneous	8 (50)
Ocular	3 (19)
Cardiovascular	3 (19)
Pericarditis	2 (13)
Increased acute-phase reactants during disease flare	10/10
Pathergy positive	3/10
Autoantibodies positive	8/14

(8/16, 50%). Less frequently, ocular (3/16, 19%) and cardiovascular involvement (3/16, 19%) was observed.

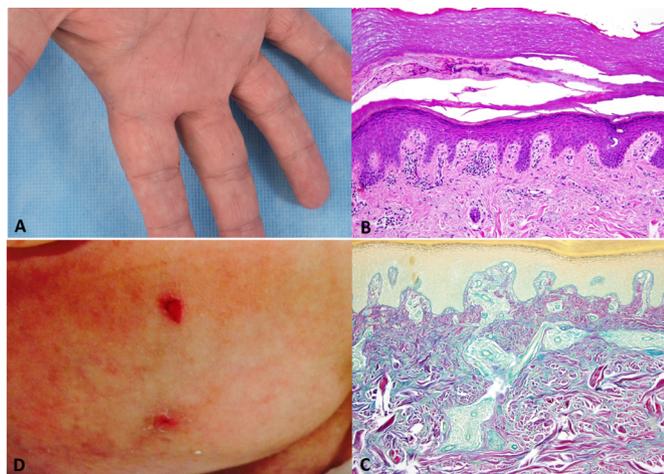
Seven patients (44%) suffered from recurrent, predominantly respiratory tract and otorhinolaryngological infections, especially during childhood. Infections were of viral and/or bacterial origin; only one of these patients was concomitantly treated with immunosuppressive agents. An overview of clinical symptoms occurring during the disease course is presented in tables 2 and 3.

### Ulcers

Recurrent painful oral, genital and/or gastrointestinal ulcers were the hallmark feature of the disease. All 16 (100%) patients developed oral ulcers, whereas genital ulcers were observed in 15/16 (94%) and gastrointestinal ulcers in 6/9 patients with gastrointestinal complaints. Ulcerations recurred frequently (every month to every few months, at various intervals), isolated or in association with other symptoms such as fever, abdominal pain and arthralgia. Singular or multiple ulcers of various sizes (0.5–2 cm) lasted about 7–10 days and some healed with scarring (oral, genital or gastrointestinal location). Oral ulceration sites included the lips, tongue, cheeks, gums and hard palate, genital ulcers developed on vulva, vagina or the scrotum, and intestinal ulcerations were observed from the oesophagus to the rectum and perineum. Most patients could not identify an underlying trigger; in one patient, oral ulcers were exacerbated by acidic food.

### Other clinical manifestations

Gastrointestinal symptoms were documented in 9/16 (56%) patients and ranged from isolated abdominal pain to severe intestinal inflammation with bowel perforation. Six (38%) patients suffered from recurrent, intermittently bloody diarrhoea. Musculoskeletal symptoms were frequent (9/16, 56%); polyarthritides was documented in seven patients, and in three of them arthritis was the initial disease manifestation. Cutaneous involvement (8/16, 50%) varied considerably and included pustular, folliculitis-like rashes, acne and dermal abscesses (figure 2). Ocular findings included severe, treatment-refractory anterior uveitis in two sisters (patients 1 and 2), as well as retinal vasculitis with chorioretinal scarring and macular fibrosis and anterior uveitis in another young girl (patient 4). Cardiovascular involvement was noted in three (19%) patients. Patient 8 was presented at age 9 months with fever and a pericardial



**Figure 2** Clinical and histological manifestations in patients with A20 haploinsufficiency (HA20). (A) Pustules and vesicles at various stages of development are seen. There is a mild desquamation and some hyperkeratosis. (B) Magnification image (20 $\times$ ) of the palmar pustule seen in (A), stained with H&E. An infiltrate containing lymphocytes and neutrophils is noted in the stratum corneum. (C) The colloidal iron staining for mucin (blue, magnification image 20 $\times$ ) shows extensive intradermal mucin accumulation. Mucin accumulation is a feature of connective tissue diseases, such as systemic lupus erythematosus, but is not characteristically seen in palmoplantar pustulosis or pustular psoriasis. This is an unusual finding and fits with the HA20 phenotype of both autoimmune and autoinflammatory manifestation. (D) Cutaneous ulcers on the buttock.

effusion, which responded well to wide-spectrum antibiotic treatment. Another patient was diagnosed with pericarditis with a large effusion possibly related to *Mycoplasma pneumoniae* infection, which relapsed after discontinuation of corticosteroids but responded to colchicine (patient 10). In addition, patient 16, an 8-year-old boy with negative antiphospholipid antibodies and unremarkable hypercoagulability screen, presented with venous thrombi at sites of previous indwelling catheters. Two months later he was diagnosed with pulmonary arterial embolisms while he had no apparent venous thrombi. It is unclear whether these were true pulmonary embolisms or thromboses resulting from pulmonary artery vasculitis. Notably, none of the other patients developed thromboses or emboli. Neurological manifestations were reported in two (13%) patients, and both were diagnosed with central nervous system (CNS) vasculitis (patient 4 based on brain imaging and patient 16 based on a frontal lobe punctate). The clinical manifestations of the individual patients are shown in table 2.

### Laboratory and histology findings

Acute-phase reactants were elevated, especially during relapses. Most patients had normal acute-phase reactants in between flares; in three patients, C reactive protein and erythrocyte sedimentation rate were persistently elevated prior to treatment response. We observed fluctuating levels of various low-titre antibodies, including antinuclear antibodies (4/10 patients tested), anti-dsDNA (2/6) and anti-Sm/RNP (2/4) antibodies. Lupus anticoagulant was positive in 6/7 patients tested and anticardiolipin antibody in 2/5 patients. Patients 11 and 12 (family 5), both of whom suffered from recurrent viral and bacterial infections, were diagnosed with unclassified immunodeficiency with IgG subclass deficiency, absent polysaccharide vaccination

response and lymphopaenia. None of the eight patients investigated for recurrent genital ulcers had evidence of herpes simplex infection.

Given clinical similarity with BD, we reviewed pathology results and found they were variable among the 10 patients tested: positive in three patients, negative in six patients and inconclusive in one patient. HLA-B51 was positive in 2/5 patients tested. Tissue biopsy of at least one site was performed in 12/16 (75%) patients; pathological findings on histology included non-specific chronic inflammation in six, findings consistent with pustules and normal results in two patients each (bone marrow aspirate and intestinal mucosa in one, and lymph node in another) (figure 2). A kidney biopsy performed in patient 5 was consistent with class V lupus nephritis with glomerular basement membrane thickening, minimal inflammatory cell infiltrate, and extensive deposition of complement and immunoglobulins. Except for the frontal lobe punctate performed in patient 16, none of the tissue samples showed evidence of vasculitis. Online supplementary table 1 summarises the laboratory and histopathological features of patients with HA20.

### Treatment

All patients required treatment. Four patients responded well to monotherapy with colchicine. The other patients were treated with a monotherapy or a combination of immunosuppressive drugs, including systemic corticosteroids, disease-modifying drugs and cytokine inhibitors (anti-tumour necrosis factor (anti-TNF), anti-interleukin-1 (IL-1), anti-IL-6). After treatment-refractory disease courses, 12 patients eventually improved on either infliximab, anakinra, tofacitinib, colchicine or methotrexate in combination with thalidomide. More recently, therapeutic approaches were based on functional cytokine studies; cytokine inhibitors such as infliximab and anakinra proved effective in suppressing systemic inflammation in 7/9 patients. Most patients responded to high-dose corticosteroids but also suffered from major side effects. Patient 4 eventually underwent autologous haematopoietic stem cell transplantation for CNS vasculitis associated with a severe, SLE-resembling condition. She went into remission for 18 months, but subsequently developed a CNS vasculitis flare, anterior uveitis, idiopathic thrombocytopenic purpura and recurrence of orogenital ulcers, for which various immunosuppressive agents were reinitiated. Previous and current treatment regimens of the individual patients are presented in table 1.

Three out of seven patients with recurrent respiratory tract and otorhinolaryngological infections underwent tonsillectomy; two patients (mother and daughter of family 5) received immunoglobulin replacement for low IgG subclass and the daughter also had repeat tympanostomy.

### Outcome/complications

HA20 disease was characterised by unprovoked episodes of inflammatory symptoms or chronic inflammation. None of the patients developed lymphoma or malignancy. One patient died from HA20. Patient 16, who presented with severe intestinal involvement and presumed small vessel CNS vasculitis, died from uncontrollable disease and upper airway obstruction due to haemorrhage following erosion of the carotid artery from extension of bilateral tonsillar ulcerations. He was anticoagulated for pulmonary arterial embolisms.

Treatment-associated complications included corticosteroid-induced side effects such as Cushing syndrome, growth retardation, vertebral compression fractures, diabetes mellitus

and arterial hypertension in five patients, and severe lymphopaenia and neutropaenia under azathioprine in another patient. Disease-associated and treatment-associated complications are shown in [table 2](#).

## DISCUSSION

Herein, we describe the clinical manifestations and disease course of 16 patients with HA20, the largest cohort to date. The disease was characterised by early-onset systemic inflammation accompanied with recurrent oral, genital and/or gastrointestinal ulcers. Other clinical manifestations and disease course varied considerably even among patients with the same mutation, and ranged from severe or fatal multisystemic inflammation to mild disease with recurrent orogenital ulcers, arthralgia and cutaneous lesions. This suggests a role of modifying alleles in the disease progression and possible contribution of environmental factors such as diet.

Recurrent painful oral, genital and/or gastrointestinal ulcers were the hallmark feature in all subjects. Besides ulcers, various other, BD-like clinical manifestations such as articular, gastrointestinal, cutaneous and ocular symptoms were notable.<sup>8–13</sup> As a consequence, the majority of patients (>70%) were initially diagnosed or suspected of having BD. HA20 is considered a monogenic type of BD due to highly penetrant novel germline mutations in *TNFAIP3* and earlier onset symptoms. Polygenic and common BD, on the other hand, typically manifests in early adulthood and does not have clear dominant inheritance. Large case series of patients with paediatric BD reported a symptom onset in later childhood, between 6.9 and 12.3 years of age<sup>14–18</sup>; however, it is not clear how many of these patients carry mutations in *TNFAIP3*. Despite some similarities with BD, we recognised several important characteristics that help differentiate HA20 from polygenic BD, as shown in [table 4](#). These other distinguishing observations in patients with HA20 included scarring oral ulcers, isolated anterior uveitis or retinal vasculitis with necrotising inflammation, recurrent fever, severe intestinal inflammation, elevated acute-phase reactants, the fluctuating presence of various autoantibodies, and a disease course refractory to standard treatment, all of which are unusual findings in typical BD.<sup>13 19</sup>

Although BD was the most common initial diagnosis, the heterogeneity of clinical phenotypes, variable temporal occurrence of symptoms, presence of various autoantibodies and

histology resulted in the diagnosis of other inflammatory and autoimmune diseases. Family 1 presented with a clinical picture resembling JIA and/or rheumatoid arthritis (RA), patient 10 with symptoms compatible with PFAPA and patient 16 with features resembling Crohn's disease. Half of the patients were found to have fluctuating autoantibodies and two sisters (patients 4 and 5) were initially diagnosed with SLE. Thus, it is likely that some patients with early-onset SLE might have mutations in *TNFAIP3*.

In addition to the 16 patients described in this study, four case reports including a total of 11 patients from four unrelated families were published in the literature since the initial publication by Zhou and colleagues.<sup>4–7</sup> HA20 was recently reported in two Japanese families with an early-onset BD-like clinical picture.<sup>4 5</sup> Similar to most patients in our cohort, these two families presented with recurrent orogenital ulcers and fevers; some family members also suffered from intestinal involvement, cutaneous lesions, nephrotic syndrome, polyarthritis or uveitis.<sup>4 5</sup> Furthermore, HA20 was reported in a Japanese patient diagnosed with autoimmune lymphoproliferative syndrome (ALPS) and in a British boy with complex autoimmunity.<sup>6 7</sup> The Japanese patient had presented with early onset, recurrent fever, bilateral cervical lymphadenopathy, hepatosplenomegaly and an extensive cutaneous rash suggestive of Kawasaki disease. Consistent with the diagnosis of ALPS, immunophenotyping revealed an increased percentage of double-negative T cells and a decrease in IgM memory B cells. However, unlike in patients with ALPS, the central memory, naïve, terminally differentiated effector memory T cells re-expressing CD45RA+, and effector memory subpopulations of CD3+ CD8+ T cells were normal in this patient.<sup>6</sup> The boy of British ancestry was investigated for a complex, treatment-refractory autoimmune syndrome characterised by insulin-dependent diabetes, cytopaenias, hepatitis, enteropathy and interstitial lung disease.<sup>7</sup> Prior to his molecular diagnosis of HA20, he underwent haematopoietic stem cell transplantation and is in complete remission (except for diabetes). He was diagnosed with a novel de novo heterozygous 2 bp deletion in *TNFAIP3*, p.V489Afs\*7 in the second zinc finger domain (ZnF2). His disease-associated variant resides in a different domain of A20 from pathogenic mutations reported in all other patients and the ZnF4 domain may have other unappreciated functions.<sup>3 7</sup> Most patients reported in our study are carriers for loss-of function protein truncating mutations in the ovarian tumour domain. This resulted in decreased deubiquitination of

**Table 4** Clinical and laboratory features that are helpful to differentiate between A20 haploinsufficiency (HA20) and Behçet disease.

Features	HA20	Behçet disease <sup>9 13 16–19</sup>
Disease onset	Mostly early childhood	Early adulthood
Inheritance	Autosomal dominant	Complex inheritance pattern with familial aggregation in up to 20% of cases
Fever	Recurrent	Usually absent
Ulcers	May heal with scarring	Usually no scarring of oral ulcers
Eyes	Severe ocular disease ▶ Anterior uveitis ▶ Retinal vasculitis and choroiditis with necrotising inflammation	▶ Posterior or panuveitis ▶ Recurrent superficial retinal infiltrates resolving within days without chorioretinal scarring ▶ Peripheral retinal occlusive periphlebitis
Gastrointestinal	(Bloody) diarrhoea	Isolated abdominal pain*
Musculoskeletal	Mostly polyarthritis	Usually oligoarthritis
Erythrocyte sedimentation rate/C reactive protein	Elevated, especially during disease relapses	Often normal
Autoantibodies	Low titre, fluctuating presence	Usually absent
Autoimmune features	Systemic lupus erythematosus-like disease and other autoimmune features possible	

\*Gastrointestinal involvement in Behçet disease is usually mild and consists essentially of abdominal pain or discomfort except for patients from Japan and Korea.

key signalling molecules, such as RIP1 and NEMO, increased activity of the NF- $\kappa$ B pathway, and high expression of proinflammatory cytokines.<sup>3,20</sup> Although the molecular aetiologies of the British boy's disease and our patients with HA20 are the same, his distinct clinical symptoms could be related to the presence of other unknown modifying gene alleles. Identification of additional HA20-associated mutations is necessary to better understand the full spectrum of phenotypes associated with the distribution of pathogenic mutations in A20.

Polymorphisms or mutations in *TNFAIP3* have been associated with many autoimmune diseases, among them JIA, RA, IBD, SLE, type 1 diabetes, psoriasis, coeliac disease and coronary artery disease.<sup>20–27</sup> In murine models, cell-specific ablation of A20 causes clinical features characteristic of these human diseases.<sup>1,20</sup> Mice with A20 deficiency in myeloid cells develop polyarthritis mimicking human RA,<sup>28</sup> while enterocyte-specific deficiency of A20 increases the susceptibility of mice to intestinal inflammation.<sup>29</sup> Tissue-specific deletion of A20 in B cells or dendritic cells leads to the production of autoantibodies and an autoimmune syndrome resembling SLE.<sup>30,31</sup> Mice deficient for A20 (A20<sup>-/-</sup>) develop severe multiorgan inflammation, cachexia and early death.<sup>32</sup> Although A20 was initially described as required for termination of TNF-induced signals, the excessive inflammation observed in double-deficient mice, A20-TNF or A20-TNFR1, suggested that A20 might be critical for the regulation of TNF-independent signals. In addition, A20 has been shown to downregulate the activity of NLRP3 inflammasome and patients with HA20 had increased activity of NLRP3.<sup>3</sup> A20 functions as a tumour suppressive gene and somatic mutations have been identified in B cell lymphomas.<sup>33</sup> None of the patients in this cohort developed lymphoma or malignancy.

In clinical practice, HA20 may be considered in patients with an early-onset, dominantly inherited inflammatory disease who present with recurrent oral and genital ulcerations, fluctuating autoantibodies, and a treatment-refractory disease course.

There was no standardised treatment in this HA20 cohort. The patients received various immunosuppressive drugs prior to the diagnosis of HA20; more recently, therapeutic approaches were guided by functional cytokine studies. Elevated levels of many proinflammatory cytokines (IL-1, TNF, IL-6, IL-18, IFN $\gamma$ , IP-10) have been documented in patients with HA20.<sup>3,20</sup> Anticytokine agents such as anti-TNF or anti-IL-1 have been effective in suppressing the systemic inflammation in most of our patients. Haematopoietic stem cell transplant might be considered in patients with severe and treatment-refractory disease.

This study is limited by its retrospective design and other biases related to which patients received testing.<sup>3</sup> Recurrent ulcers and a Behçet-like disease were characteristic features in this cohort, likely reflecting bias in which patients are screened for HA20. However, ulcers of variable extension and severity were documented in index cases and in all affected family members, suggesting that ulcers may be the hallmark feature of the disease. Given the retrospective study design and the disease pleiotropy, it is unlikely that HA20 is the unique underlying cause for all disease manifestations. The increased use of diagnostic whole exome sequencing will help identify other contributing disease modifying rare or common variants.<sup>34</sup>

In conclusion, HA20 disease in this cohort was characterised by early-onset inflammation and recurrent oral, genital and/or gastrointestinal ulcers and often positive family history. Other disease features and disease course varied considerably with an overall high morbidity and mortality. Treatment regimens should be based on severity of inflammatory manifestations, and often consist of targeted therapy with cytokine inhibitors to control

the inflammation. In the future, identification of patients with monogenic inflammatory diseases will be important to understand the pathophysiology and guide treatments for common rheumatological diseases.

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**Acknowledgements** We thank the patients and their families for participating in this study. We thank Ingrid Goh for the technical support. The authors acknowledge Dres Chyi-Chia R Lee and Ed Cowen for their valuable assistance in the management of the cases.

**Contributors** FAA and RML: coordination of the study, data collection and analysis, manuscript preparation. EDB, SWC, EG, AG, PH, HLL, SO, DMS, DLS, AvR-K, IA and DLK: data collection and analysis, revision of the manuscript for important intellectual content. All authors approved the final version to be published.

**Funding** FAA was supported by grants from the Rhyner-Bangert Foundation, Starr-Foundation, Swiss League against Rheumatism, Foundation W1, Alberta Children's Hospital Research Institute Foundation and SickKids Foundation.

**Competing interests** None declared.

**Ethics approval** The study was approved by the Institutional Research Ethics Board (REB 1000053697).

**Provenance and peer review** Not commissioned; externally peer reviewed.

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#### REFERENCES

- Catrysse L, Vereecke L, Beyaert R, et al. A20 in inflammation and autoimmunity. *Trends Immunol* 2014;35:22–31.
- Wertz IE, O'Rourke KM, Zhou H, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF- $\kappa$ B signalling. *Nature* 2004;430:694–9.
- Zhou Q, Wang H, Schwartz DM, et al. Loss-of-function mutations in *TNFAIP3* leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. *Nat Genet* 2016;48:67–73.
- Ohnishi H, Kawamoto N, Seishima M, et al. A Japanese family case with juvenile onset Behçet's disease caused by *TNFAIP3* mutation. *Allergol Int* 2017;66:146–8.
- Shigemura T, Kaneko N, Kobayashi N, et al. Novel heterozygous C243Y A20/*TNFAIP3* gene mutation is responsible for chronic inflammation in autosomal-dominant Behçet's disease. *RMD Open* 2016;2:e000223.
- Takagi M, Ogata S, Ueno H, et al. Haploinsufficiency of *TNFAIP3* (A20) by germline mutation is involved in autoimmune lymphoproliferative syndrome. *J Allergy Clin Immunol* 2017;139:1914–22.
- Duncan CIA, Dinnigan E, Theobald R, et al. Early-onset autoimmune disease due to a heterozygous loss-of-function mutation in *TNFAIP3* (A20). *Ann Rheum Dis* 2017;annrheumdis-2016-210944.
- Koné-Paut I, Darce-Bello M, Shahram F, et al. Registries in rheumatological and musculoskeletal conditions. Paediatric Behçet's disease: an international cohort study of 110 patients. One-year follow-up data. *Rheumatology* 2011;50:184–8.
- Nanthapaisal S, Klein NJ, Ambrose N, et al. Paediatric Behçet's disease: a UK tertiary centre experience. *Clin Rheumatol* 2016;35:2509–16.
- Koné-Paut I, Gorchakoff-Molinas A, Weschler B, et al. Paediatric Behçet's disease in France. *Ann Rheum Dis* 2002;61:655–6.
- Mora P, Menozzi C, Orsoni JG, et al. Neuro-Behçet's disease in childhood: a focus on the neuro-ophthalmological features. *Orphanet J Rare Dis* 2013;8:18.
- Uluduz D, Kürtüncü M, Yapıcı Z, et al. Clinical characteristics of pediatric-onset neuro-Behçet disease. *Neurology* 2011;77:1900–5.
- Saadoun D, Wechsler B. Behçet's disease. *Orphanet J Rare Dis* 2012;7:20.

- 14 Atmaca L, Boyvat A, Yalçındağ FN, *et al.* Behçet disease in children. *Ocul Immunol Inflamm* 2011;19:103–7.
- 15 Karıncaoğlu Y, Borlu M, Toker SC, *et al.* Demographic and clinical properties of juvenile-onset Behçet's disease: A controlled multicenter study. *J Am Acad Dermatol* 2008;58:579–84.
- 16 Koné-Paut I, Shahram F, Darce-Bello M, *et al.* Consensus classification criteria for paediatric Behçet's disease from a prospective observational cohort: PEDBD. *Ann Rheum Dis* 2016;75:958–64.
- 17 Koné-Paut I, Yurdakul S, Bahabri SA, *et al.* Clinical features of Behçet's disease in children: an international collaborative study of 86 cases. *J Pediatr* 1998;132:721–5.
- 18 Krause I, Uziel Y, Guedj D, *et al.* Childhood Behçet's disease: clinical features and comparison with adult-onset disease. *Rheumatology* 1999;38:457–62.
- 19 Koné-Paut I. Behçet's disease in children, an overview. *Pediatr Rheumatol Online J* 2016;14:10.
- 20 Aksentijevich I, Zhou Q. NF- $\kappa$ B Pathway in autoinflammatory diseases: dysregulation of protein modifications by ubiquitin defines a new category of autoinflammatory diseases. *Front Immunol* 2017;8:399.
- 21 Plenge RM, Cotsapas C, Davies L, *et al.* Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. *Nat Genet* 2007;39:1477–82.
- 22 Thomson W, Barton A, Ke X, *et al.* Rheumatoid arthritis association at 6q23. *Nat Genet* 2007;39:1431–3.
- 23 Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78.
- 24 Fung EY, Smyth DJ, Howson JM, *et al.* Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. *Genes Immun* 2009;10:188–91.
- 25 Nair RP, Duffin KC, Helms C, *et al.* Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* 2009;41:199–204.
- 26 Boonyasrisawat W, Eberle D, Bacci S, *et al.* Tag polymorphisms at the A20 (TNFAIP3) locus are associated with lower gene expression and increased risk of coronary artery disease in type 2 diabetes. *Diabetes* 2007;56:499–505.
- 27 Trynka G, Zhernakova A, Romanos J, *et al.* Coeliac disease-associated risk variants in TNFAIP3 and REL implicate altered NF-kappaB signalling. *Gut* 2009;58:1078–83.
- 28 Matmati M, Jacques P, Maelfait J, *et al.* A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. *Nat Genet* 2011;43:908–12.
- 29 Vereecke L, Sze M, Mc Guire C, *et al.* Enterocyte-specific A20 deficiency sensitizes to tumor necrosis factor-induced toxicity and experimental colitis. *J Exp Med* 2010;207:1513–23.
- 30 Chu Y, Vahl JC, Kumar D, *et al.* B cells lacking the tumor suppressor TNFAIP3/A20 display impaired differentiation and hyperactivation and cause inflammation and autoimmunity in aged mice. *Blood* 2011;117:2227–36.
- 31 Hövelmeyer N, Reissig S, Xuan NT, *et al.* A20 deficiency in B cells enhances B-cell proliferation and results in the development of autoantibodies. *Eur J Immunol* 2011;41:595–601.
- 32 Lee EG, Boone DL, Chai S, *et al.* Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 2000;289:2350–4.
- 33 Ma A, Malynn BA. A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat Rev Immunol* 2012;12:774–85.
- 34 Demirkaya E, Zhou Q, Smith CK, *et al.* Brief report: deficiency of complement 1r subcomponent in early-onset systemic lupus erythematosus: the role of disease-modifying alleles in a monogenic disease. *Arthritis Rheumatol* 2017;69:1832–9.