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ORIGINAL RESEARCH

Clinical phenotype and laboratory markers in patients affected by haploinsufficiency of A20 (HA20): a case series from two Italian centres

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ABSTRACT

Introduction Haploinsufficiency of A20 (HA20) is a monogenic disease caused by heterozygous *TNFAIP3* variants. Despite the marked clinical variability, no genotype–phenotype correlation or validated laboratory biomarkers have been identified so far. Neurobehavioural abnormalities have been reported in murine models, but their prevalence in humans remains unclear.

Objectives To describe a cohort of patients with HA20 from two centres, evaluating age-related clinical variability; to assess the prevalence of neuropsychiatric symptoms; to explore the inflammatory profile of the patients, including interferon (IFN)-γ-inducible chemokines (CXCL9/10) and type 1 IFN signature (IS), as well as the therapies administered.

Methods Clinical and laboratory data of 17 subjects from six families heterozygous for *TNFAIP3* variants (American College of Medical Genetics and Genomics class 4–5) were retrospectively collected. Disease activity, treatments, CXCL9/10 and IS levels were collected. Continuous variables were expressed as medians (IQR). Age at onset was compared using the Kruskal-Wallis test, and groups were compared through the Wilcoxon test or Fisher's exact test.

Results Clinical manifestations included oral aphthosis (88%), recurrent fever (53%), gastrointestinal inflammation (53%), autoimmunity (47%), genital ulcers (47%), neuropsychiatric symptoms (41%), arthritis/tenosynovitis (18%) and skin inflammation (12%). Disease onset before 5 years of age was associated with a higher prevalence of neuropsychiatric symptoms during lifetime (p=0.004), whereas arthritis was more common in patients with later onset. The median age at onset differed significantly according to the type of clinical manifestation (p<0.001). Higher IS levels were found in patients with active disease (p<0.01).

Conclusions HA20 shows marked clinical heterogeneity both between and within families. Clinical manifestations appear age-related and early disease onset was associated with increased neuropsychiatric involvement lifetime. Finally, type 1 IS may represent a potential biomarker of disease activity in HA20.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Haploinsufficiency of A20 (HA20) is a monogenic disease, caused by heterozygous *TNFAIP3* variants, characterised by marked clinical heterogeneity, with both autoinflammatory and autoimmune manifestations.

⇒ Neurobehavioural symptoms have been described in murine *TNFAIP3* models, but their prevalence in humans remains unknown.

WHAT THIS STUDY ADDS

⇒ Clinical manifestations in HA20 appear to be age-related, with autoinflammatory features predominating in early childhood and autoimmune features in adolescence and adulthood.

⇒ Early disease onset is associated with increased lifetime neuropsychiatric involvement, which is underestimated.

⇒ Higher levels of type 1 interferon signature (IS) were found in patients with active disease.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Careful reconstruction of patients' clinical history over time, including neuropsychiatric symptoms, is essential, as HA20 should be considered at any age.

⇒ Type 1 IS may help assess disease activity in patients with HA20.

INTRODUCTION

Haploinsufficiency of A20 (HA20) is a monogenic inflammatory disease caused by heterozygous variants in *TNFAIP3*, encoding the deubiquitinase A20, a key negative regulator of multiple innate and adaptive immune signalling pathways.[1 2] A20 controls Nuclear factor kappa-B (NF-κB)-mediated responses, as well as other proinflammatory cascades, and its disruption

confers a high risk of both autoinflammatory and autoimmune manifestations. In keeping with this broad biological role, HA20 is associated with striking clinical heterogeneity, both between and within families, suggesting a contribution of additional genetic, epigenetic and environmental modifiers.[2] Several authors tried to identify a genotype–phenotype association, but results have been inconsistent. Chen *et al*[3] proposed that the affected protein domain could determine the clinical phenotype. However, this was not confirmed in a more recent case series of 177 patients presenting with different clinical phenotypes and with the disruption of the same A20 domain.[4] In fact, A20 was shown to downregulate the activity of NLRP3 inflammasome in murine models[5 6] and Zhou *et al*[7] demonstrated increased NLRP3 activity in the peripheral blood mononuclear cells of patients with HA20. Moreover, A20 not only inhibits Tumor necrosis factor (TNF)-dependent NF-κB activation, but also NF-κB activation in response to interleukin (IL)-1, CD40, IL-17, the signalling through pattern recognition receptors (PRRs) and T-cell and B-cell antigen receptor activation. Also, A20 can have distinct functions in different cell types. Murine studies have shown that mice deficient in A20 died prematurely due to hyperinflammatory severe multiorgan failure, whereas mice with tissue-specific A20 deletion showed different clinical manifestations depending on the cell type affected.[1] Such murine models may better reflect the clinical heterogeneity observed in vivo. The literature provides several series of patients with HA20 presenting with both autoinflammatory clinical pictures characterised by recurrent fever, Behçet-like oral and/or genital aphthosis, inflammatory bowel disease (IBD), and autoimmune conditions such as juvenile idiopathic arthritis (JIA), rheumatoid arthritis, systemic lupus erythematosus (SLE), type 1 diabetes, thyroiditis and coeliac disease.[8 9] A20 disruption in mice has been linked to neuroinflammation and to a variety of neurobehavioural manifestations.[10–12] Heterozygous A20-deficient mice have been considered a useful model for neuropsychiatric SLE (NPSLE) by Deams *et al*[12] considering the high prevalence of behavioural abnormalities in females, the worsening of phenotypes with age and after exposure to lipopolysaccharide intracerebroventricular injection. However, data on neurological and/or behavioural features of patients with HA20 are scarce. High circulating levels of interferon (IFN)-γ-inducible chemokines (CXCL9/10) have been previously described in a family with HA20,[8] but no laboratory markers of the disease have been currently defined so far.

The aim of this study was to present a case series of patients with HA20 from two centres, focusing on the age-related evolution of clinical manifestations; to assess the prevalence of neuropsychiatric symptoms in this cohort; to explore the inflammatory profile of the patients, including IFN-γ-inducible chemokines (CXCL9/10) and type 1 IFN signature (IS), as well as the therapies administered.

METHODS

This is a retrospective observational study performed between January 2024 and December 2025 at the Bambino Gesù Children's Hospital, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy, in collaboration with the Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, Italy. Eligible patients were subjects with a genetically confirmed diagnosis of HA20, followed at the two centres. Patients carrying American College of Medical Genetics and Genomics (ACMG) class 3 variants, despite a compatible clinical phenotype, were excluded. 17 patients from 6 families with heterozygous likely loss-of-function variants in *TNFAIP3* (ACMG class 4–5) were finally included.

Patients of family 1 (pt1, pt2, pt3, pt4) have been previously described.[8] These individuals are included here within an expanded cohort and reanalysed with updated clinical data and extended observation in order to assess age-related phenotypic evolution.

Clinical and laboratory data were retrospectively collected from patients' medical records and anonymised prior to analysis. We summarised demographic and clinical characteristics of each patient, including age, sex, age at onset, history of recurrent fever, oral or genital ulcers, skin lesions, musculoskeletal, gastrointestinal symptoms, ocular manifestations, neuropsychiatric symptoms, history of autoimmunity, as well as the treatment received.

Neuropsychiatric disorders were diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria. The need for psychological support was assessed during routine visits, and specific tests were administered according to psychological evaluation when needed (eg, Patient Health Questionnaire-9) for depressive symptoms, Generalized Anxiety Disorder-7 for anxiety).[13 14]

For the purpose of age-stratified analyses, patients were divided according to age at first disease manifestation (<5 years vs ≥5 years). This cut-off was defined a priori to distinguish early childhood, a developmental phase characterised by heightened innate immune responsiveness, from later stages of immune maturation. This age threshold is also consistent with the typical age at onset of periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA), supporting the biological rationale for this stratification.[15]

Laboratory data included erythrocyte sedimentation rate (ESR), C reactive protein (CRP), immunoglobulin levels, lymphocyte subset and inflammatory biomarkers. Data availability varied among patients due to the retrospective nature of the study. Circulating levels of CXCL9/10 and/or IS were measured at diagnosis and, when available, during the disease course (often during disease flares), in the absence of clinical evidence of infection. Longitudinal measurements were not systematically available for all patients. At each control visit, the disease activity was assessed using a Physician's Global Assessment (PGA) Scale, based on a 5-point scoring system (0=inactive, 1=minimal, 2=mild, 3=moderate and 4=severe) that included fever, increase of inflammatory markers (CRP ≥0.5 mg/dL and ESR ≥15 mm/hour),

presence of pain and/or functional impairment and physician Visual Analogue Scale modified from what is described in Pardeo *et al*.[16] To calculate the IFN Score, real-time PCR was used to analyse the relative expression of six Interferon-Stimulated Genes (Interferon alpha-inducible protein 27, Interferon-induced protein 44-like, Interferon-induced protein with tetratricopeptide repeats 1, ubiquitin-like modifier, Radical S-adenosyl methionine domain containing 2 and Sialic acid-binding Ig-like lectin 1), normalising the quantities of target genes with the expression levels of two housekeeping genes, *HPRT1* and *GAPDH*, as previously described.[17] Relative quantitative gene expression analysis was conducted using the 2^{-DDCt} method compared with the control group. The threshold value for determining positivity or negativity was determined by the mean of the IS of healthy subjects +2 SD and was set at >2.

Next-Generation Sequencing was performed on patients' and, when possible, parents' genomic DNA on a NovaSeq6000 platform (Illumina). The reads were aligned to human genome build GRCh37/UCSC hg19. The Dragen pipeline and the Geneyx software LifeMap Sciences were respectively used for the variant calling and variant annotation. Global minor allele frequency for analysed variants was calculated according to Genome Aggregation Database. The variants were evaluated by VarSome[18] and categorised in accordance with the ACMG recommendations.[19]

Continuous variables were shown as medians and IQR (Q1–Q3), median ages of symptom onset were compared through Kruskal-Wallis test and groups were compared using the Wilcoxon test or Fisher's exact test, as appropriate. Statistical analyses were performed using Jamovi software (V.2.3.28.0; The Jamovi Project, 2022). All statistical tests were two tailed, and p values<0.05 were considered statistically significant.

RESULTS

All 17 patients were European and carried truncating variants resulting in stop codon (ACMG class 4–5), one of which consisting in a de novo copy number variation (deletion of exons 7–9) resulting in A20 loss of function.[20] All but one variants were inherited in an autosomal dominant pattern. Only one of such variants (pArg183Ter) was located in the OTU domain, which mediates the deubiquitylation activity for k63-linked ubiquitin chains, while the other five variants mainly affect the zinc-finger domains, which recognise K63-linked ubiquitin chains (figure 1).

Genetic, clinical and laboratory characteristics of the whole cohort are reported in online supplemental file 1. Family pedigrees are shown in online supplemental file 2. Patients' clinical features and their respective age at onset are reported in table 1. The median age at first symptom onset varied significantly according to the type of clinical manifestation (p<0.001, Kruskal-Wallis test, figure 2), with oral aphthosis and recurrent fever manifesting at a younger age and arthritis/tenosynovitis manifesting at a later age.

Seven patients presented neuropsychiatric symptoms with an overall prevalence of 41%. Six patients had clinical onset <5 years and presented more frequently neuropsychiatric symptoms during lifetime (p=0.004, Fisher's exact test), while patients with later onset presented more often arthritis/tenosynovitis (figure 3). Among these, two patients received a diagnosis of attention-deficit/hyperactivity disorder (ADHD) at 8 years according to DSM-5 (p7,

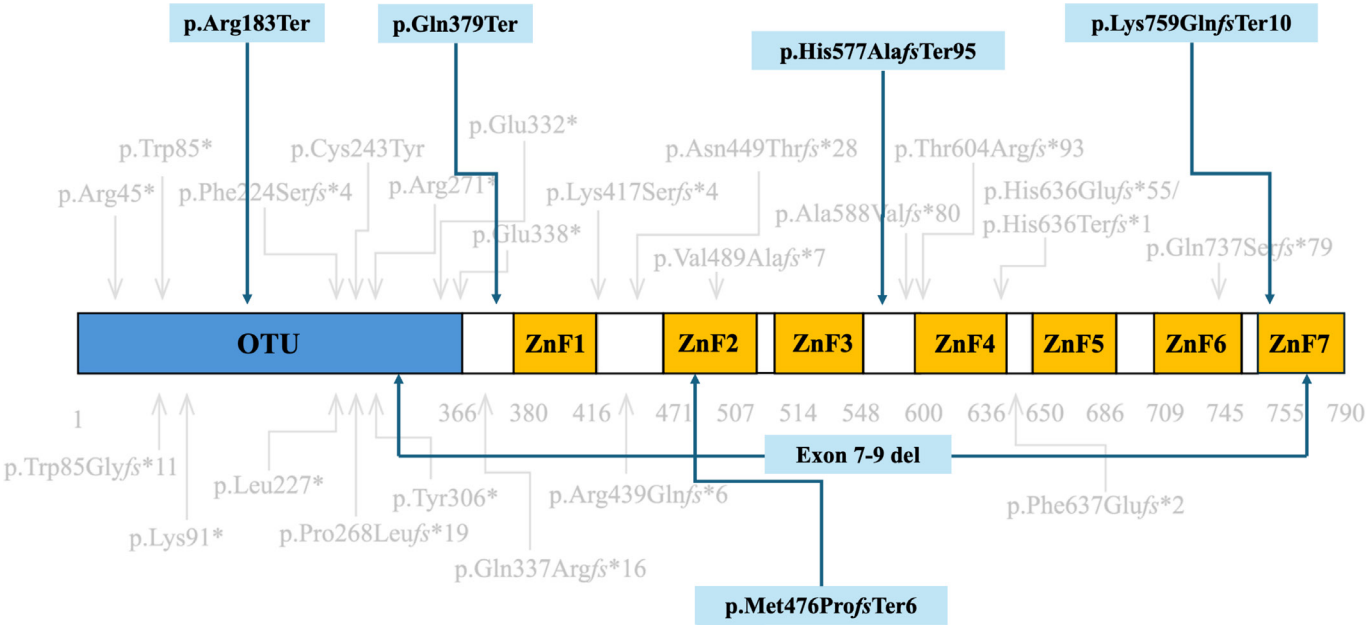

Figure 1 TNFAIP3 variants of the six families (American College of Medical Genetics and Genomics class 4–5). OTU, ovarian tumor-type deubiquitinase domain.

Table 1 Patients' clinical features and their respective age at onset

	N (%)	Age of symptom onset Median (Q1–Q3)	Specific manifestations (No of patients)
Number of patients	17 (100)		
Female sex	12 (71)		
Age at onset		5 (3–6)	
Recurrent fever	9 (53)	4 (2–6)	
Oral aphthosis	15 (88)	5 (4.5–6)	
Genital ulcers	8 (47)	5.5 (3.8–7.8)	
GI involvement	9 (53)	9 (8–9.5)	IBD-like disease: ileitis/colitis (5), gastritis/ duodenitis (3), oesophagitis (1)
Skin involvement	2 (12)	11.5 (10.8-12.3)	Hidradenitis (1), urticarial rash (1)
Arthritis/tenosynovitis Arthralgia	3 (18) 7 (41)	19 (14-22) 17 (12-20)	
Neuropsychiatric symptoms	7 (41)	10 (8–13.5)	Mood disorders (3), anxiety (2), ADHD (2), pica (1), oppositional defiant disorder (1)
Autoimmune manifestations (at least one)	8 (47)	10 (8–22)	Coeliac disease (4), DMT1 (1), Hashimoto thyroiditis (7), autoimmune haemolytic anaemia (1), autoimmune hepatitis (1), RA/SS (1) atrophic autoimmune gastritis (2)
Laboratory immune dysregulation	3 (18)		Hypogammaglobulinaemia (1), hypergammaglobulinaemia (3), CD4/CD8 ratio<1 (2), low RTE (2), high DNT (1)

ADHD, attention deficit hyperactivity disorder; DMT1, type1 diabetes mellitus; DNT, double negative T cells; GI, Gastrointestinal; IBD, intestinal bowel disease; RA, rheumatoid arthritis; RTE, recent thymic emigrants; SS, Sjogren syndrome.

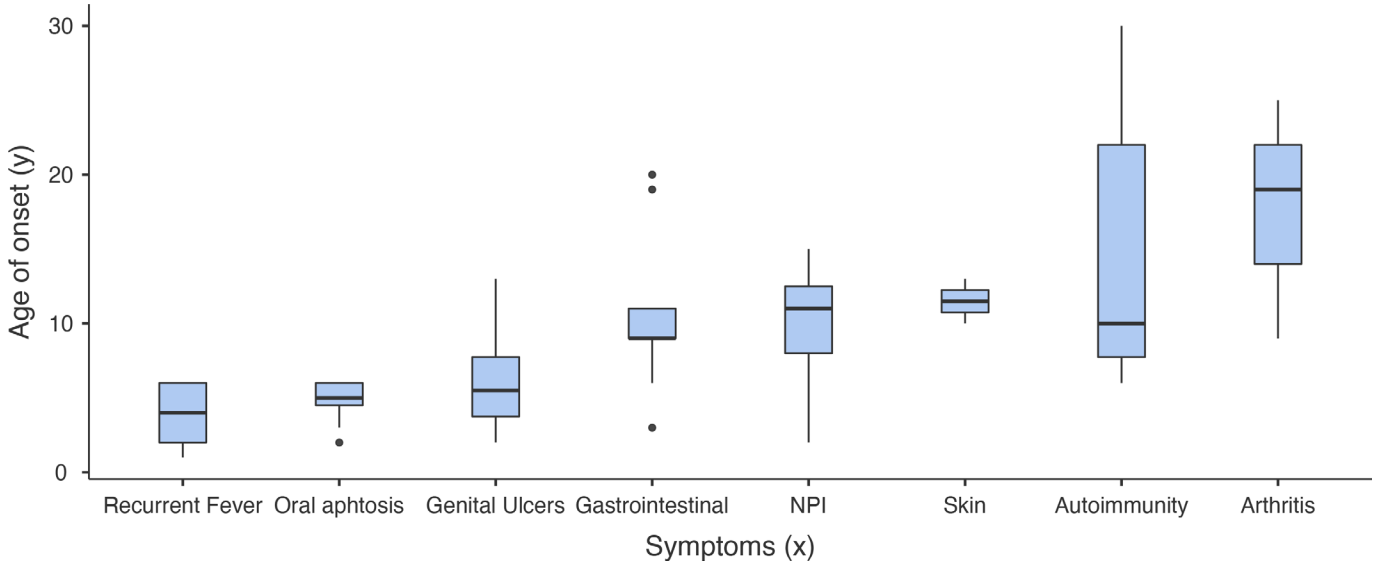

Figure 2 Representation of the median ages of onset (y) of specific clinical features (x) reported by patients with haploinsufficiency of A20. Medians are significantly different among age groups (p<0.001, Kruskal-Wallis test). The black dots represent the outliers. NPI, neuropsychiatric involvement.

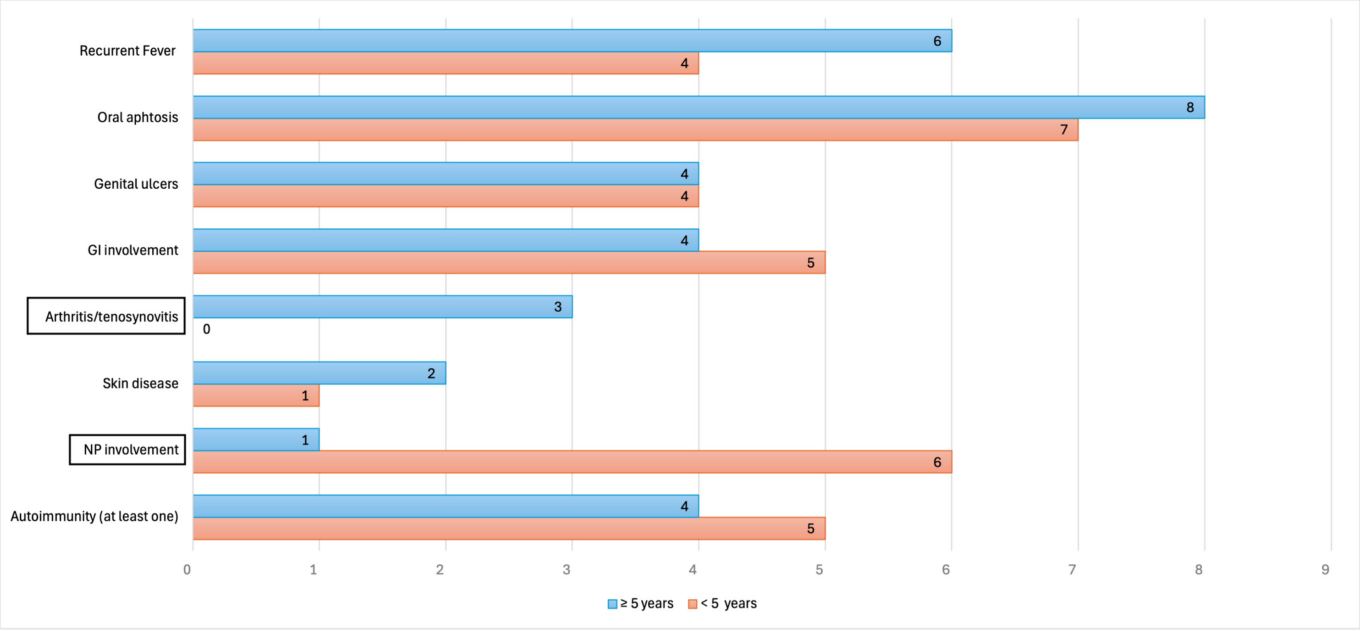

Figure 3 Clinical characteristics of patients during lifetime depending on age at onset of any clinical features of haploinsufficiency of A20. Neuropsychiatric symptoms were more frequent in patients with disease onset before 5 years of age (p=0.004, Fisher's exact test). GI, gastrointestinal; NP, neuropsychiatric.

p14); two developed anxiety disorder during adolescence for which follow-up is still ongoing (p7, p10); one developed disruptive mood dysregulation disorder (p10); another patient was diagnosed with oppositional defiant disorder (pt13) and one with pica (pt15). Pt16 and pt17 developed depression during adolescence which persisted during adult age, leading in one case to suicide attempt.

Data on therapies are detailed in online supplemental file 3. TNF inhibitors were used in seven patients (7/17), including etanercept, adalimumab and golimumab. Methotrexate was prescribed in five patients (5/17), and Janus kinase (JAK) inhibitors in three subjects (2 upadacitinib and 1 tofacitinib). Other therapies included colchicine (3/17), apremilast (2/17), hydroxychloroquine (2/17), mesalazine (1/17), intravenous immunoglobulin (1/17), leflunomide (1/17) and thalidomide (1/17).

Possible associations were assessed between the clinical phenotype and the inflammatory profile including IFN-γ-inducible chemokines (CXCL9/10) values or type 1 IS. CXCL9/10 values did not differ significantly according to the type of variant or the clinical phenotype (data not shown). However, higher levels of IS (median 14.3, IQR 6.9–20.8) were found among patients with active disease (PGA≥1) when compared with inactive disease (median 2.1, IQR 1.6–3.1) (Mann-Whitney, Wilcoxon, p<0.01).

DISCUSSION

This case series highlights a recurring pattern of age-related clinical presentations in HA20, with autoinflammatory features predominating in early childhood and autoimmune manifestations in adolescence and adulthood. In particular, PFAPA-like symptoms and Behçet-like phenotypes were most often recognised in the first years of age, IBD-like phenotype typically develop during school age, whereas articular involvement tends to predominate during adolescence and later stages (including arthritis, tenosynovitis and arthralgia). Autoimmune features show greater heterogeneity in their timing, although they have been more commonly observed from school age onwards. Although the sample size is limited, these observations support the concept that HA20 behaves as a dynamic disease in which the clinical phenotypes evolve over time, likely reflecting age-dependent changes in immune maturation superimposed on impaired A20-mediated regulation.[21 22] The predominance of recurrent fever and mucosal inflammation at younger ages may reflect the relative dominance of innate immune response in early life, whereas the later emergence of arthritis and other autoimmune features may coincide with the progressive maturation and diversification of adaptive/humoral immunity.

This proposed immunological trajectory is consistent with age-related differences in cytokine production, including the delayed achievement of adult levels expression of multiple cytokines and type 1 IFN responses. Adaptive/humoral immunity may play a major role at older ages, likely driven by cumulative exposure to pathogens and antigens (eg, infections). Such physiological variations in immune responses and cytokine production across different ages may be amplified by insufficient A20 regulation, leading to a more autoinflammatory or autoimmune phenotype. For example, monocytes produce adult levels of certain cytokines, such as TNF-α and IL-6, by around 3 years of age, whereas others, including IL-12 and IFN-γ, remain at lower levels until approximately 13

years of age.[23] A similar difference has been described for the IFN-α/β response which is lower in the toddler than in adolescent.[24] In their case series of patients with HA20, Kadowaki *et al*[25] showed a marked differentiation of TH17 cells with ageing, which are known to play a relevant role in the development of autoimmunity.[26] This is in line with previous reports on heterozygous *TNFAIP3* knockout mice, where reduced A20 expression led to the accumulation of germinal centres B cells and IgG autoantibodies with age.[27 28]

Several case series of patients with HA20 have been reported in the literature. Yu *et al*[21] described the clinical phenotypes of 61 patients from 26 families with several different diagnoses received by the same subjects during lifetime (ranging from PFAPA symptoms more often in the early infancy, to Behçet disease, to JIA and Crohn disease later in time). The same was reported by Aeschlimann *et al*[10 22] in their cohort of 16 patients, where different features were noticed across members of the same family and different diagnoses were made depending on age. A very broad spectrum of phenotypes ranging from Behçet disease, to SLE, to lymphoproliferation and immune dysregulation with recurrent infections, have been reported by Karri *et al*[29] in subjects with HA20, reflecting the pleiotropic cytokine dysregulation typical of this disease.

Such an intrafamilial phenotypic heterogeneity has been attributed to the role of other modifying alleles acting in disease progression, to different tissue-specific A20 deficiency and to epigenetic modifications and environmental factors such as diet, infections and vaccinations. Another possible explanation may lie in the broad age range of patients included in previously reported case series. Reanalysing these cohorts by considering the age at onset of individual clinical manifestations might help to better delineate the age-dependent evolution of the phenotype.

Neuropsychiatric involvement emerged as a notable feature in our cohort, with a spectrum encompassing ADHD, anxiety, mood dysregulation, oppositional defiant disorder, pica and depressive symptoms, including one suicide attempt. The overall prevalence of neuropsychiatric symptoms in this series was higher than previously reported (accounting around 40%), and patients with disease onset before 5 years of age more frequently developed neuropsychiatric manifestations during their lifetime.

Central nervous system inflammation has been reported with variable incidence in literature. In a large case series of 177 patients described by Elhani *et al*[4] the prevalence was around 10%. Several data are available on animal studies, where *TNFAIP3* mutant mice are prone to severe neuroinflammation, highlighting the critical role for A20 in the control of microglia activation and regulation of neuronal synaptic function.[11 30] Moreover, variants in A20 have been implicated as predisposing factor for NPSLE both in murine models[12] and in humans, as suggested by the identification of a de novo *TNFAIP3* frameshift variant reported by Duan *et al*[31] in a patient with NPSLE. In particular, the authors proposed a pathogenic model involving blood–brain barrier dysfunction linked to impaired A20 deubiquitinating activity, together with increased microglial cytokine production as an additional contributor to neuroinflammation.[31] Intriguingly, progressive neuroinflammation has been described in a patients with a T674P variant in *TNFAIP3*, who was treated with the JAK inhibitor baricitinib and showed marked clinical, radiologic and immunologic improvement.[32] Subsequently, four additional patients with NPSLE were reported in a cohort of 16 patients with HA20, previously diagnosed with SLE, although no psychiatric manifestations were described in that series.[9] Although these observations might suggest that neuropsychiatric involvement in HA20 could occur in the context of NPSLE, none of the patients in our cohort fulfilled the classification criteria for SLE (neither 2012 Systemic Lupus International Collaborating Clinics classification criteria nor 2019 European Alliance of Associations for Rheumatology/American College of Rheumatology classification criteria).[33]

Given the small sample size, the lack of a control group and the high background prevalence of anxiety and depression in young people with chronic disease, these findings should be considered hypothesis generating rather than providing precise estimates of the risk.[34 35] Nonetheless, when viewed alongside experimental data showing *TNFAIP3* mutant mice are prone to severe neuroinflammation and behavioural alterations, and reports implicating *TNFAIP3* variants in NPSLE, our results support the need for systematic assessment of neuropsychiatric symptoms in patients with HA20. Also, it is noteworthy that five of the seven patients with neuropsychiatric involvement in this series belong to the same family, which might raise the hypothesis of a variant-specific effect. However, the same *TNFAIP3* variant was previously reported in a family with relatively mild clinical manifestations, apart from the proband who presented with cutaneous disease.[36] This discrepancy highlights once again the marked phenotypic variability associated with HA20 and may reflect incomplete penetrance or the influence of additional genetic and environmental modifiers. The possible contribution of familial neuropsychiatric comorbidities independent of HA20 should also be considered.

Finally, although no specific laboratory biomarker profile was found in this cohort of patient, IS was found significantly elevated in patients with active disease (PGA≥1), suggesting IS as a good biomarker of disease activity. These data confirm what was recently reported by Shiraki *et al*[37] and this is the first demonstration of such an association in a western population. Elevated levels of many proinflammatory cytokines (IL-1, TNF, IL-6, IL-18 and IFN-γ) have been recognised in patients with HA20.[2 7] Our group previously described an increase in circulating levels of IFN-γ-inducible chemokines (CXCL9/10) in a family.[8] High levels of CXCL9 were also found in this

cohort, although no statistically significant associations were found when trying to correlate the values with disease activity and specific features of the disease. It is already known that A20 not only acts as the major negative regulator of NF-κB-induced inflammation, but that it is also involved in controlling other immunological pathways including necroptosis, inflammasome activation, JNK signalling and JAK-signal transducer and activator of transcription signalling.[29] The reason why some patients have higher levels of type 2 IFN-induced biomarkers is still not explained and further studies are needed to understand if any correlation exists between such biomarkers and different clinical phenotypes and response to treatment.

This study has several limitations, including its retrospective design, the relatively small number of patients and the absence of a control group, all of which limit the ability to draw causal inferences or fully account for potential confounders. The findings on age-related phenotypic clustering and neuropsychiatric involvement therefore need validation in larger, ideally multicentre cohorts with standardised assessments and longitudinal follow-up.

In summary, the extreme variability of clinical manifestations and their age-dependent expression suggest that HA20 should be considered at any age, particularly in patients with coexisting autoinflammatory and autoimmune features. Careful reconstruction of the patients' clinical history over time, including neuropsychiatric symptoms, and the integration of type 1 IS measurements may help in diagnosis, monitoring and treatment, although larger studies are needed to confirm these findings.

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Contributors Study design: ATo and AI. Data acquisition: SF and LDN. Data interpretation: LDN, SF, AP and EDM. Manuscript drafting: LDN and SF. CPa and CPe performed the genetic analyses. GP, VM, IC, MG, EDM, ATe and AP performed laboratory tests for biomarker measurement. CC, SP and MB contributed to patient care and data interpretation. ATo, FDB and AI critically revised the manuscript for important intellectual content. All authors approved the final version of the manuscript. LDN accepts full responsibility for the work and acts as guarantor of the study.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. The study was approved by the ethics committee of the Bambino Gesù Children's Hospital (3086 OPBG 2023), and written informed consent was obtained from patients and/or their parents. Participants gave informed consent to participate in the study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. Additional data are available from the corresponding author upon reasonable request.

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