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Chronic hepatic involvement in the clinical spectrum of A20 haploinsufficiency

Samuel DESHAYES[1,2]*, Céline BAZILLE[3]*, Elma EL KHOURI[4], Isabelle KONE-PAUT[5], Irina GIURGEA[4], Sophie GEORGIN-LAVIALLE[6], Nicolas MARTIN SILVA[1], Anaël DUMONT[1,2], Isabelle OLLIVIER[7], Serge AMSELEM[4], Hubert de BOYSSON[1,2], and Achille AOUBA[1,2]

[1] Department of Internal Medicine, CHU de Caen Normandie, 14000 Caen, France

[2] Normandie Univ, UNICAEN, 14000 Caen, France

[3] Department of Pathology, CHU de Caen Normandie, 14000 Caen, France

[4] Department of Genetics, Sorbonne Université, UPMC University Paris 06, hôpital Trousseau, AP–HP, 75012 Paris, France

[5] Department of Pediatric Rheumatology, CEREMAIA, Bicêtre Hospital, AP-HP, University of Paris Sud Saclay, AP-HP, Le Kremlin-Bicêtre, France

[6] Department of Internal Medicine, Sorbonne Université, UPMC University Paris 06, CEREMAIA, hôpital Tenon, AP–HP, 75020 Paris, France

[7] Department of Hepatogastroenterology, CHU de Caen Normandie, 14000 Caen, France

* These authors contributed equally to this study

Corresponding authors:
Prof. Achille AOUBA
Service de Médecine Interne et d'Immunologie Clinique, CHU Côte de Nacre – Université Basse Normandie, Avenue de la Côte de Nacre, 14000 CAEN, FRANCE

Email: aouba-a@chu-caen.fr

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List of abbreviations:

HA20: A20 haploinsufficiency

TNFAIP3*:* tumor necrosis factor-alpha induced protein 3

NF-κB: nuclear factor kappa light chain enhancer of activated B cells

IL: interleukin

NLRP3: nucleotide-binding domain and leucine-rich repeats containing pyrin domain

NGS: next-generation sequencing

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Abstract

Background & Aims: Secondary to *TNFAIP3* mutations, A20 haploinsufficiency (HA20) is a recently described autoinflammatory disease with clinical features similar to those of Behçet's and Crohn's diseases but with a constantly expanding clinical spectrum. Here, we describe HA20 liver involvement in three new patients from the same family.

Methods: We retrospectively assessed clinical, biological and/or histological findings for 8 patients over three generations of the same family with heterozygous mutations in the *TNFAIP3* gene (c.259C>T, p.Arg87*).

Results: The 8 patients exhibited the following: aphthous ulcers (8/8, bipolar in 7), autoimmune features (6/8, including 5 with definitive autoimmune disease diagnoses, i.e., type I diabetes, Hashimoto thyroiditis, pernicious anemia, and/or 5 with antinuclear antibodies ≥320), pustulosis/folliculitis (5/8), abdominal pain (4/8), arthralgia (3/8), enlarged cervical lymph nodes (3/8) and pericarditis (1/8). In addition, three patients (twin sisters and their grandmother aged 23 and 70 years, respectively) exhibited persistent mild hepatic cytolysis associated with splenomegaly (n=3), hepatomegaly (n=1) and/or liver atrophy (n=1) on echography. We could not detect any other causes of chronic liver diseases. Liver biopsies from 3 patients displayed hepatic fibrosis, hepatocyte injury and/or $CD4^+$/$CD8^+$ T lymphocyte infiltration, and patterns of inflammatory cells and NLRP3 or NF-κB immunostaining differed from the predominant neutrophil infiltration observed in skin or some digestive tract biopsies.

Conclusions: This study reinforces the dual involvement of innate and adaptive immunity in HA20 according to both acute and chronic injury and the organ involved and widens its clinical spectrum to include chronic hepatic involvement.

Keywords: A20 haploinsufficiency; *TNFAIP3*; hepatic fibrosis; oral and genital aphthosis

Lay summary

A20 haploinsufficiency (HA20) is a recently described autoinflammatory disease that combines features of deregulation of both innate and adaptive immunity. HA20 was associated with chronic liver involvement secondary to hepatic fibrosis, hepatocyte injury and/or inflammatory T lymphocyte infiltrates with moderate NF-κB and/or NLRP3 staining. Chronic hepatic involvement should probably be searched for in HA20 patients, and HA20 should be suspected in patients with familial chronic hepatitis

Introduction

A new autoinflammatory disease related to a dominant mutation in the *tumor necrosis factor-alpha induced protein 3* (*TNFAIP3*) gene and the consequent haploinsufficiency of the encoded A20 protein (also known as TNFAIP3) was recently described in 2016 by Zhou *et al*.[1] These authors reported 6 unrelated families who exhibited an autosomal dominant disease with clinical features mimicking Behçet's disease.[1] Since then, fewer than 100 cases have been published, and almost all were previously misdiagnosed as Behçet's or Crohn's disease. Indeed, all three entities share hallmark signs such as mucocutaneous (oral and/or genital ulcers, cutaneous (pseudo)folliculitis/pustulosis), digestive, ocular (uveitis/retinal vasculitis) and rheumatologic (arthralgia/arthritis) signs.[2–5] However, the clinical spectrum of this newly described entity is constantly evolving, and HA20 combines features of deregulation of both innate and adaptive immunity. Indeed, significant frequencies of generalized lymphadenopathies, enlarged tonsils, hepatosplenomegaly, autoimmune lymphoproliferative syndrome, autoimmune diseases such as Hashimoto disease, insulin-dependent diabetes, lupus nephritis and autoantibodies have been observed in HA20 patients.[2,4,6,7] Additionally, *TNFAIP3* polymorphisms have been associated with several autoimmune diseases in genome-wide association studies, such as systemic lupus erythematosus,[8–10] rheumatoid arthritis[11,12] and psoriasis,[13] which also share some clinical and histopathological features with HA20.

Although rarely reported, some HA20 patients develop liver or lung fibrosis.[6,14,15] The mechanisms underlying fibrosis with respect to overactivation of either innate or adaptive immunity are not clear, and the respective role of HA20 itself requires further investigation.

We observed chronic hepatic involvement in three of eight patients in a HA20 French family. After ruling out the usual causes, we attempted to link liver involvement to the HA20 spectrum. In addition, we report liver histopathologic findings and compare them with skin and digestive histology in these 3 patients.

Methods

Human subjects

Eight Caucasian patients from three generations in the same family were diagnosed with HA20 and evaluated at the Department of Internal Medicine of Caen University Hospital, France.

Ethics

This study was conducted in compliance with good clinical practices and the Declaration of Helsinki principles. In accordance with French public health law (Art. L 1121-1-1, Art. L 1121-1-2), written consent from the patient is not required for this type of study. The local ethics committee of Caen University Hospital confirmed the observational non-interventional retrospective nature of our study (Comité Local d'Ethique de la Recherche en Santé, n°1708).

Gene sequencing

Molecular diagnosis was performed in the Laboratory of Genetics, Sorbonne Université, Hôpital Trousseaux, Paris, France.

Genomic DNA was isolated from peripheral leukocytes using standard procedures. We used next-generation sequencing (NGS) with custom sequence capture (Nimblegen SeqCap EZ Choice system; Roche Sequencing, Pleasanton, California, USA) of the exons and flanking intronic sequences of the following genes implicated in autoinflammatory diseases: *MEFV*, *MVK*, *NLRP3*, *NLRC4*, *TNFAIP3*, and *TNFRSF1A*. Sequencing was performed using the MiSeq or NextSeq500 (Illumina, San Diego, California, USA) platform according to the manufacturer's instructions. The conventional bioinformatics pipeline was used as previously described.[16] All results obtained by NGS for each family proband were further confirmed by Sanger sequencing.

Sanger sequencing confirmed the identified mutation in all symptomatic family members, whereas the results were concordantly negative in subjects without clinical or biological signs, including hepatic involvement. Exons and flanking intronic sequences were PCR amplified from genomic DNA. The primer sequences used are available on request. The PCR products were sequenced using the Big Dye Terminator sequencing kit (Applied Biosystems, Foster City, California, USA) with a 96-capillary ABI Prism sequencer and were then analyzed using SeqScape V.2.6 software (Applied Biosystems).

Histological characterization

Three patients with persistent hepatic cytolysis underwent liver and digestive system biopsies. In addition, one patient underwent skin biopsy twice for rashes. The biopsy samples were fixed in formalin and embedded in paraffin. Four-micrometer sections were generated and stained with hematoxylin-eosin-saffron. An immunohistochemical study was carried out using an automated immunohistochemistry system with antibodies against the following: CD20 (L26, Dako, 1/20e), CD3 (polyclonal rabbit, Dako, 1/100e), CD4 (4B12, Leica, 1/40e), CD8 (4B11, Leica, 1/50e), NLRP3 (HPA012878, Atlas antibodies, 1/400e), IL-1β (3A6, Cell Signaling, 1/100e), and NF-κB p65 (D14E12, Cell Signaling, 1/1500e). Briefly, 4-µm sections were prepared from paraffin blocks and placed on Superfrost plus slides. After antigen unmasking with target retrieval solution, citrate pH 6 or 9 was added according to the antibodies to be used, and the immunohistochemical study was carried out by an immunohistochemistry automaton (Ventana Benchmark XT). After incubation with the primary antibody (20 to 60 min), detection was carried out with an Ultraview Universal DAB Detection Kit (Dako) indirect detection kit according to the supplier's instructions.

A biopsy sample of chronic active colitis was used as an immunohistochemical control for some antibodies.

Results

Clinical presentations

In this study, 8 patients, all women, harboring the *TNFAIP3* gene c.259C>T p.(Arg87*) mutation from 3 generations of a nonconsanguineous Caucasian family were evaluated. The clinical presentations included the following (Figure 1, Supplementary Table 1): aphthous ulcers (8/8, bipolar in 7), autoimmune features (6/8, including 5 patients with definitive autoimmune diseases, i.e., type I diabetes, Hashimoto thyroiditis and pernicious anemia, and/or 5 with antinuclear antibodies ≥320), pustulosis/folliculitis (5/8), recurrent abdominal pain (4/8), arthralgia (3/8), cervical enlarged lymph nodes (3/8) and pericarditis (1/8). None had uveitis. The severity and burden of disease differed for each patient.

In addition, three patients, including 23-year-old twin sisters (P5, P6) and a 70-year-old grandmother (P1), exhibited chronic (> 6 months) hepatic cytolysis (fluctuating ASAT and/or ALAT levels from 1-6 times the normal values) associated with splenomegaly (P1, P5 and P6), hepatomegaly (P1) and/or liver atrophy (P6) on echography. Because persistent hepatic abnormalities occurred in 3/8 of the HA20-affected family members, we performed extensive exploratory work-up to exclude other causes of chronic hepatic cytolysis, namely, hemochromatosis, environmental, toxic, viral, autoimmune, metabolic and iatrogenic causes. P6 exhibited a decrease, followed by normalization, in her liver biological tests under treatment with steroids and azathioprine and then adalimumab.

Anatomical pathology

Liver involvement

Liver biopsies performed for P1, P5 and P6 revealed variable but also found common findings. Portal tracts were either normal (P1), enlarged by fibrous septa (P6) or had prominent fibrous

septa surrounding regeneration nodules, favoring a diagnosis of cirrhosis (P5). For P6, the transjugular liver biopsy obtained was too small to definitively confirm cirrhosis. All biopsies presented minimal to severe lymphocytic inflammatory infiltrate in portal tracts with slight involvement of the bordering plate, suggestive of interface hepatitis (P1, P5 and P6). However, no plasma cells were observed. The lymphocytic infiltrate predominated around the interlobular bile ducts without lesions of lymphocytic cholangitis. All biopsy fragments contained discrete neogenesis of bile ducts, but we could not identify cholestasis or biliary thrombi. In all specimens, moderate lymphocytic inflammatory infiltration was detected within the sinusoids. Regarding immunohistochemical analysis, the inflammatory infiltrate was found to involve portal tracts and lobules and was rich in $CD3^+$ T lymphocytes in association with a few rare $CD20^+$ B lymphocytes (Figure 2). The $CD4^+$ and $CD8^+$ T cell distribution was equivalent (data not shown). Cytoplasmic and rarely nuclear labeling of portal, periportal and intrasinusoidal lymphocytic inflammatory infiltrates was observed when using the anti-NF-κB p65 antibody. However, labeling with the anti-IL-1β antibody was negative on all hepatic sections, despite the positive external control involving chronic active colitis sections. Slight nuclear labeling of intraportal, sinusoidal lymphocytes and a few nuclei of the interlobular bile ducts was observed with the anti-NLRP3 antibody (Figure 3).

Digestive tract involvement

Gastric biopsies were available for two patients (P1 and P6). P1's biopsy showed chronic atrophic gastritis in the fundus consistent with a diagnosis of pernicious anemia. The other patient had lesions of chronic nonactive interstitial gastritis but with no evidence of *Helicobacter pylori*. Lymphocyte infiltrate in the lamina propria was abundant with reactive lymphoid follicles, but no lymphocytic gastritis was present. According to immunohistochemistry, the inflammatory infiltrate was rich in T and B lymphocytes. No IL-1β

and NF-κB p65 labeling was found, and rare lymphocytes were marked with the anti-NLRP3 antibody (data not shown).

P6, who presented with episodic abdominal pain without diarrhea, underwent endoscopic examination that revealed esophageal varices and a single ulcer of the terminal ileum. Ileal biopsies showed nonspecific ulceration containing neutrophils and an intense focal or diffuse inflammatory infiltrate in the lamina propria associated with many lymphoid follicles, but typical architectural aspects of Crohn's disease were not seen. This inflammatory infiltrate contained B and T lymphocytes with an equivalent distribution of $CD4^+$ and $CD8^+$ T lymphocytes (data not shown). Anti-NLRP3 antibody staining indicated marked inflammatory cells in the lamina propria that was equivalent to a control case of active chronic colitis (Figure 4). On the other hand, there was no evidence of overexpression when using the anti-IL-1β antibody or NF-κB p65 nuclear labeling, in contrast to our chronic active colitis control sample.

Skin biopsies

Patient P1, who experienced two episodes of skin rashes, underwent two biopsies. The first involved chronic pruritic erythema with a crumbled edge at the neckline. The biopsy sample showed discrete nonspecific superficial dermatitis with an inflammatory lymphocytic infiltrate around the dermal vessels. $CD3^+$ T lymphocytes were predominant with an equivalent distribution of $CD4^+$ and $CD8^+$ lymphocytes based on immunohistochemistry (Figure 5). No expression of IL-1β, NF-κB p65 or NLRP3 was observed (data not shown). The second biopsy was performed on nonspecific pruritic erythematous patches of the forearm and elbows, which showed a diffuse polymorphic dermal infiltrate rich in polynuclear neutrophils with predominant perisweat gland localization. Immunohistochemical analysis showed slight labeling of lymphocytes with NLRP3 but no labeling with IL-1β or NF-κB p65 was found (Figure 5).

Discussion

The concomitant finding of, on the one hand, HA20 in 8 patients from three generations of the same family and, on the other hand, liver involvement in 3 of them, allowed us to include fibrotic chronic hepatitis within the spectrum of organ involvement of the disease. It should be noted that other known causes of hepatitis were ruled out based on clinical, biological and histological features. HA20 pathophysiology appears to be due to direct and/or indirect activation of both innate and adaptive immunity, which emphasizes its multicytokine background. Indeed, the coexistence of inflammatory T lymphocytes and neutrophil infiltrates in biopsy samples together with the clustering of patients with autoimmunity in this family and in those from the literature agree with this phenomenon.[2,5]

There are few reports to date on HA20-associated liver involvement. We identified in the literature only 12 other patients, aged from 3 weeks to 43 years (median: 7.5 years), from 11 unrelated families with these unique signs (Supplementary Table 2).[4–7,14,15,17–21] Only 4 of these 12 patients underwent liver biopsy, with the following histological features: isolated fibrosis (n=1) or associated with major inflammatory infiltrate (n=1);[6,15] interface hepatitis with spotty focal necrosis associated with giant multinucleated hepatocytes and marked $CD8^+$ T cell infiltration (n=1);[7] and steatosis with iron overload in Kupffer cells and sinusoidal macrophages (n=1).[14] Thus, a fully and well-characterized histological diagnosis of cirrhosis was found in only one of our patients. Although the amount of hepatic fibrous involvement differed, periportal fibrosis with constant $CD4^+$/$CD8^+$ lymphocyte inflammatory infiltration and moderate expression of NLRP3 and NF-κB was a hallmark feature. Chen *et al*. recently reviewed previously reported HA20 cases and found that patients with liver injury had either mutations in the carboxy-terminal zinc finger (ZnF) coding regions or nonsense or frameshift mutations in the amino-terminal ovarian tumor (OTU) coding region that impaired the function of both the OTU and the ZnF domains, as in our patients.[22] When examining gastric samples,

we observed nonspecific chronic gastritis or autoimmune chronic gastritis; both acute neutrophilic ulcerative colitis and chronic $CD4^+/CD8^+$ lymphocytic colitis with moderate expression of NLRP3 coexisted in colon samples. Similarly, in dermic biopsies, superficial chronic lymphocytic inflammatory dermatitis with weak expression of NLRP3 was associated with acute episodes of neutrophilic dermatitis. These histological findings favor acute attacks of lesions in a chronic inflammatory background.

The extension of $CD4^+/CD8^+$ lymphocyte inflammatory infiltrates into both histological periportal and sinusoidal areas in our biopsy samples and the marked infiltration of $CD8^+$ T cells in the case reported by Takagi *et al*.[7] indicate the predominant role of adaptive immunity overactivation in the pathophysiology of liver involvement. Of note, liver involvement seems to respond to immunosuppressive treatment.[7,15,18] The involvement of adaptive immunity in this autoinflammatory disease is also supported by the clustering of HA20 with autoimmune diseases or autoantibodies[2,5] and the efficacy of anti-TNF-α and anti-IL-6 treatments, which exhibit inhibitory actions on both innate and adaptive immunity. In addition, A20 is constitutively expressed in T and B cells.[23] A20-deficient dendritic cells show enhanced expression of costimulatory molecules and proinflammatory cytokines, which are responsible for the proliferation and hyperactivation of $CD4^+$ and $CD8^+$ cells, a decrease in regulatory T cells and spontaneous B cell activation and proliferation with features of autoimmunity in mice.[24,25] Furthermore, A20-deficient T cells in mice fail to terminate TNF-induced NF-κB responses and therefore exhibit an activated phenotype,[26] and A20-deficient $CD8^+$ T cells have increased antigen sensitivity with enhanced production of IL-2 and interferon-γ.[27] In HA20 patients, $CD4^+$ T cells are preferentially polarized toward Th9 and Th17 cells.[1,5] On the other hand, A20 in B cells prevents autoimmunity. Indeed, in mice, A20-deficient B cells produce autoantibodies and glomerular deposits.[23] Similarly, HA20 patients frequently have autoantibodies and/or autoimmune diseases.[1,2,5]

Conclusion

To conclude, this study describes 3 new HA20 patients from the same family who exhibit, in addition to several other autoimmune/inflammatory features, chronic liver involvement secondary to hepatic fibrosis, hepatocyte injury and/or inflammatory T lymphocyte infiltrates with moderate NF-κB and/or NLRP3 staining. HA20 should be searched for in patients with chronic hepatitis, especially in a family context. Our data widen the clinical spectrum of this rare and recently described disease to a chronic hepatic involvement, showing other clinical and pathophysiological aspects of HA20 probably have yet to be discovered.

References

1. 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.

2. Aeschlimann FA, Batu ED, Canna SW, et al. A20 haploinsufficiency (HA20): clinical phenotypes and disease course of patients with a newly recognised NF-kB-mediated autoinflammatory disease. *Ann Rheum Dis*. 2018;77:728–735.

3. Berteau F, Rouviere B, Delluc A, et al. Autosomic dominant familial Behçet disease and haploinsufficiency A20: A review of the literature. *Autoimmun Rev*. 2018;17:809–815.

4. Tsuchida N, Kirino Y, Soejima Y, et al. Haploinsufficiency of A20 caused by a novel nonsense variant or entire deletion of TNFAIP3 is clinically distinct from Behçet's disease. *Arthritis Res Ther*. 2019;21:137.

5. Kadowaki T, Ohnishi H, Kawamoto N, et al. Haploinsufficiency of A20 causes autoinflammatory and autoimmune disorders. *J Allergy Clin Immunol*. 2018;141:1485-1488.e11.

6. Li G-M, Liu H-M, Guan W-Z, Xu H, Wu B-B, Sun L. Expanding the spectrum of A20 haploinsufficiency in two Chinese families: cases report. *BMC Med Genet*. 2019;20:124.

7. 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;139:1914–1922.

8. Graham RR, Cotsapas C, Davies L, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat Genet*. 2008;40:1059–1061.

9. Musone SL, Taylor KE, Lu TT, et al. Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus. *Nat Genet*. 2008;40:1062–1064.

10. Adrianto I, Wen F, Templeton A, et al. Association of a functional variant downstream of TNFAIP3 with systemic lupus erythematosus. *Nat Genet*. 2011;43:253–258.

11. Plenge RM, Cotsapas C, Davies L, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. *Nat Genet*. 2007;39:1477–1482.

12. Thomson W, Barton A, Ke X, et al. Rheumatoid arthritis association at 6q23. *Nat Genet*. 2007;39:1431–1433.

13. Stuart PE, Nair RP, Tsoi LC, et al. Genome-wide Association Analysis of Psoriatic Arthritis and Cutaneous Psoriasis Reveals Differences in Their Genetic Architecture. *Am J Hum Genet*. 2015;97:816–836.

14. Hautala T, Vähäsalo P, Kuismin O, et al. A Family With A20 Haploinsufficiency Presenting With Novel Clinical Manifestations and Challenges for Treatment. *J Clin Rheumatol*. 2020. doi:10.1097/RHU.0000000000001268

15. Schwartz DM, Blackstone SA, Sampaio-Moura N, et al. Type I interferon signature predicts response to JAK inhibition in haploinsufficiency of A20. *Ann Rheum Dis*. 2020;79:429–431.

16. Jeanson L, Thomas L, Copin B, et al. Mutations in *GAS8* , a Gene Encoding a Nexin-Dynein Regulatory Complex Subunit, Cause Primary Ciliary Dyskinesia with Axonemal Disorganization: HUMAN MUTATION. *Human Mutation*. 2016;37:776–785.

17. Duncan CJA, Dinnigan E, Theobald R, et al. Early-onset autoimmune disease due to a heterozygous loss-of-function mutation in TNFAIP3 (A20). *Ann Rheum Dis*. 2018;77:783–786.

18. Gans MD, Wang H, Moura NS, Aksentijevich I, Rubinstein A. A20 Haploinsufficiency Presenting with a Combined Immunodeficiency. *J Clin Immunol*. 2020;40:1041–1044.

19. He T, Huang Y, Luo Y, et al. Haploinsufficiency of A20 Due to Novel Mutations in TNFAIP3. *J Clin Immunol*. 2020;40:741–751.

20. Kim HY, Song JY, Kim WI, et al. The First Case of an Infant with Familial A20 Haploinsufficiency in Korea. *J Korean Med Sci*. 2020;35:e252.

21. Taniguchi K, Inoue M, Arai K, et al. Novel TNFAIP3 microdeletion in a girl with infantile-onset inflammatory bowel disease complicated by a severe perianal lesion. *Hum Genome Var*. 2021;8:1.

22. Chen Y, Ye Z, Chen L, et al. Association of Clinical Phenotypes in Haploinsufficiency A20 (HA20) With Disrupted Domains of A20. *Front Immunol*. 2020;11:574992.

23. Tavares RM, Turer EE, Liu CL, et al. The ubiquitin modifying enzyme A20 restricts B cell survival and prevents autoimmunity. *Immunity*. 2010;33:181–191.

24. Song X-T, Evel-Kabler K, Shen L, Rollins L, Huang XF, Chen S-Y. A20 is an antigen presentation attenuator, and its inhibition overcomes regulatory T cell-mediated suppression. *Nat Med*. 2008;14:258–265.

25. Kool M, van Loo G, Waelput W, et al. The ubiquitin-editing protein A20 prevents dendritic cell activation, recognition of apoptotic cells, and systemic autoimmunity. *Immunity*. 2011;35:82–96.

26. 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;289:2350–2354.

27. Giordano M, Roncagalli R, Bourdely P, et al. The tumor necrosis factor alpha-induced protein 3 (TNFAIP3, A20) imposes a brake on antitumor activity of CD8 T cells. *Proc Natl Acad Sci USA*. 2014;111:11115–11120.

Figure legends

Figure 1. Pedigree of a nonconsanguineous French family with A20 haploinsufficiency. *WT: wild-type.*

Figure 2. Histological appearance of liver biopsies from patients (P1, P5 and P6) with A20 haploinsufficiency (Hematoxylin-eosin-saffron histological sections, A: magnification x4, B and D: x40, C: x20). A: Portal fibrosis with fibrous septa (stars), with periportal inflammation (arrow) (P5). B: Portal inflammation with discrete interface hepatitis (P1). C: Portal (star) and sinusoidal inflammatory infiltrate (arrow) (P1). D: Magnification of the sinusoid lymphocytic inflammatory infiltrate (arrows) (P6).

Figure 3. Immunohistochemical results for liver biopsies from patients with A20 haploinsufficiency. A: Portal inflammatory infiltrate labeled with anti-CD3 antibodies (x20) (P1). B: Portal inflammation with discrete interface hepatitis marked by anti-CD3 antibodies (x40) (P6). C: Portal inflammatory infiltrate (star) and interlobular bile ducts (arrow) marked by anti-NLRP3 antibodies (x40) (P6). D: Portal inflammatory infiltrate labeled with anti-NF-κB antibodies (x40) (P1).

Figure 4. Endoscopic, histological and immunohistochemical aspects of colonic biopsies from a patient with A20 haploinsufficiency (P6). A: Superficial ulceration of the terminal ileum. B: Superficial ulceration of the rectal mucosa with neutrophils in the crypt epithelium (inset) (HES, x10 and x40). C: Increased focal inflammatory infiltration of the lamina propria. (HES, x10). D: Infiltration of lymphocytes labeled with anti-CD3 antibodies (x20). E: Chronic inflammatory infiltration of the lamina propria without architectural disturbances (HES, x20). F: Immunohistochemical study with anti-NLRP3 antibodies in the same region as in C.

Figure 5. Histological results for cutaneous biopsies from a patient with A20 haploinsufficiency (P1). A: Superficial dermatitis with inflammatory lymphocyte infiltration around vessels (HES, x20). Lymphocytes (inset). B: $CD3^+$ lymphocytes around vessels (arrow). (x10). C: Superficial dermatitis with acute inflammatory polymorphous infiltrate around sweat glands (arrow), (HES, x20). Neutrophilic infiltrate (inset). D: NLRP3 labeling in dermal lymphocytes (same case as C, x40).

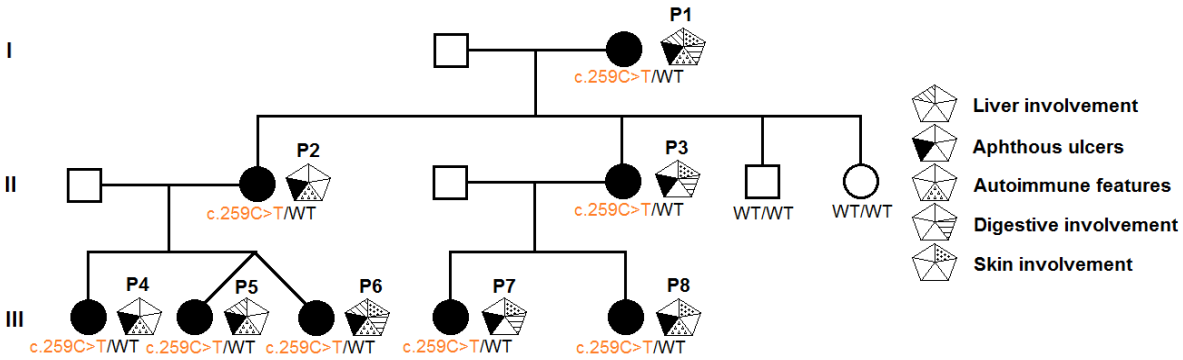

I
II
III
P1
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P2
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P3
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WT/WT
P4
c.259C>T/WT
P5
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P6
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P7
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P8
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Liver involvement
Aphthous ulcers
Autoimmune features
Digestive involvement
Skin involvement

Figure 1

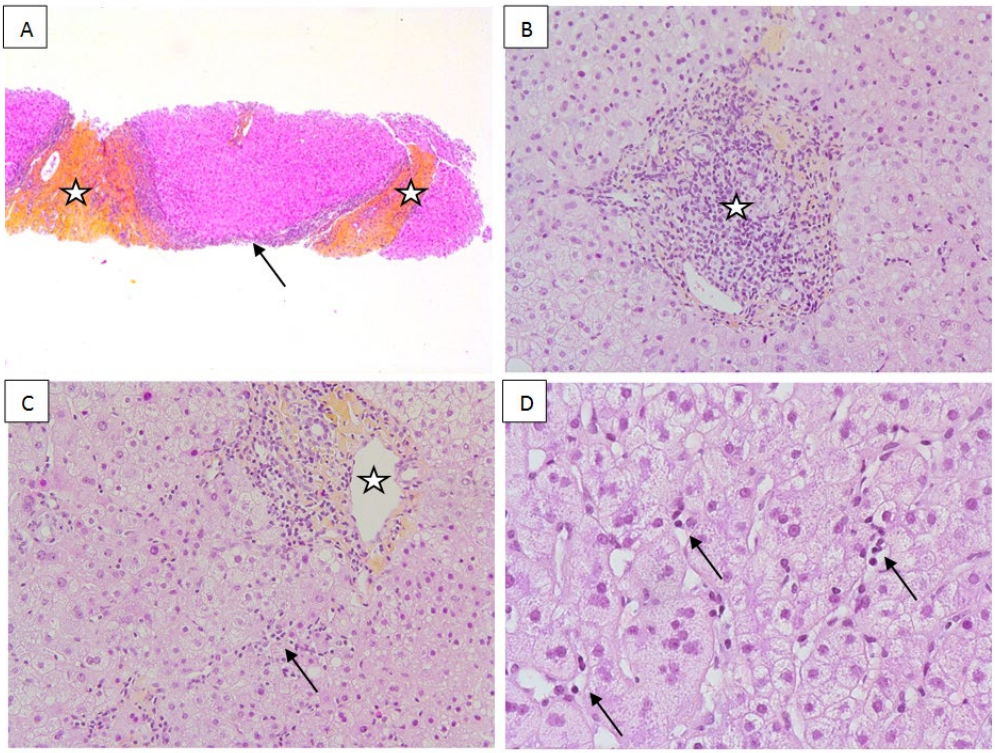


Figure 2

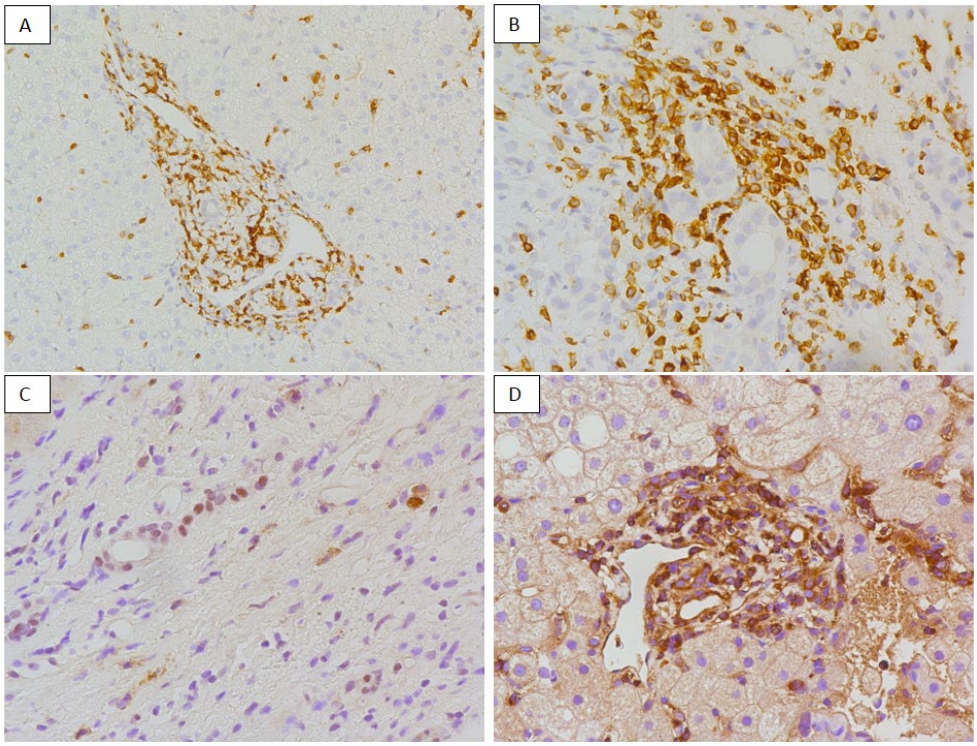


Figure 3

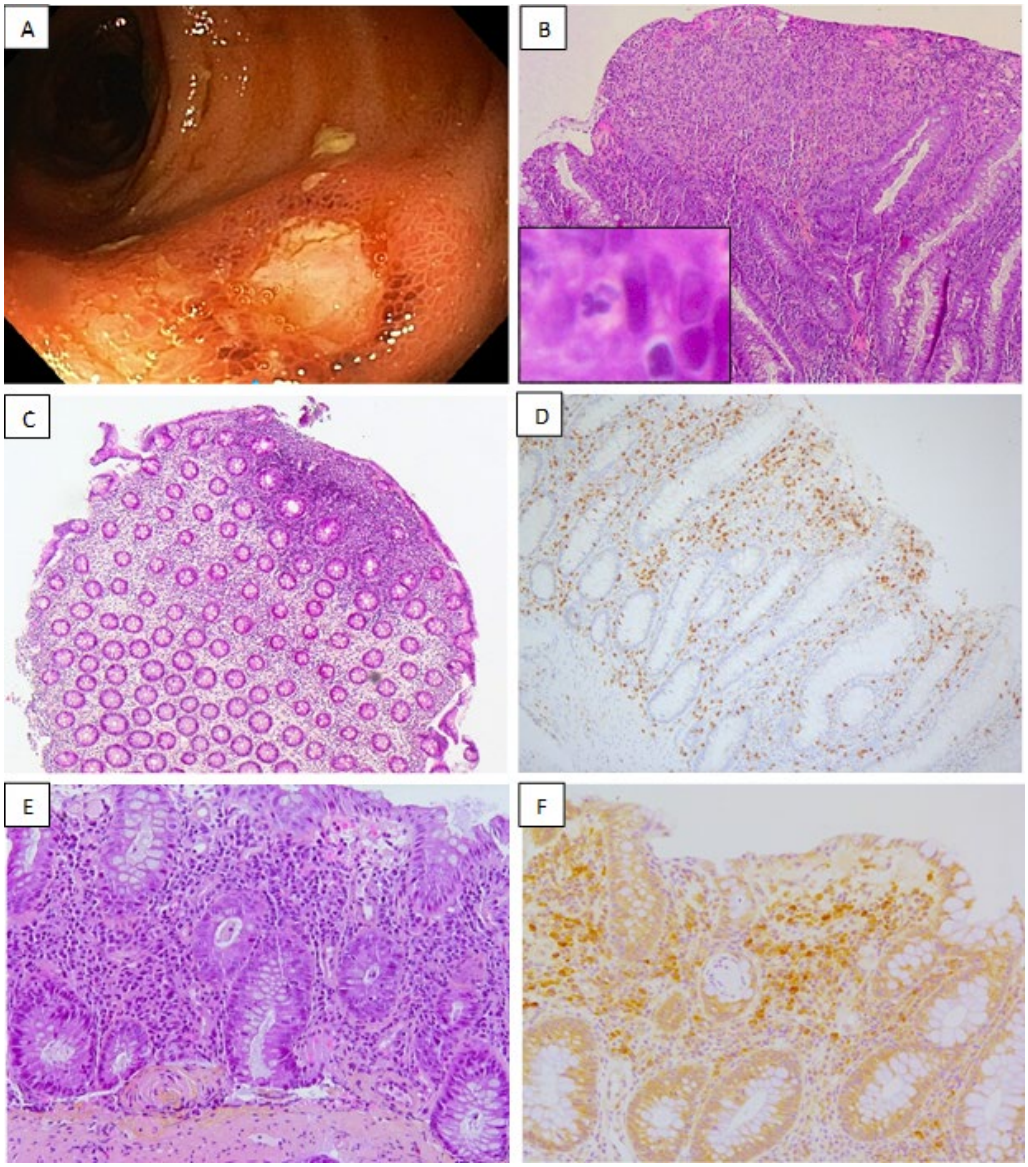


Figure 4

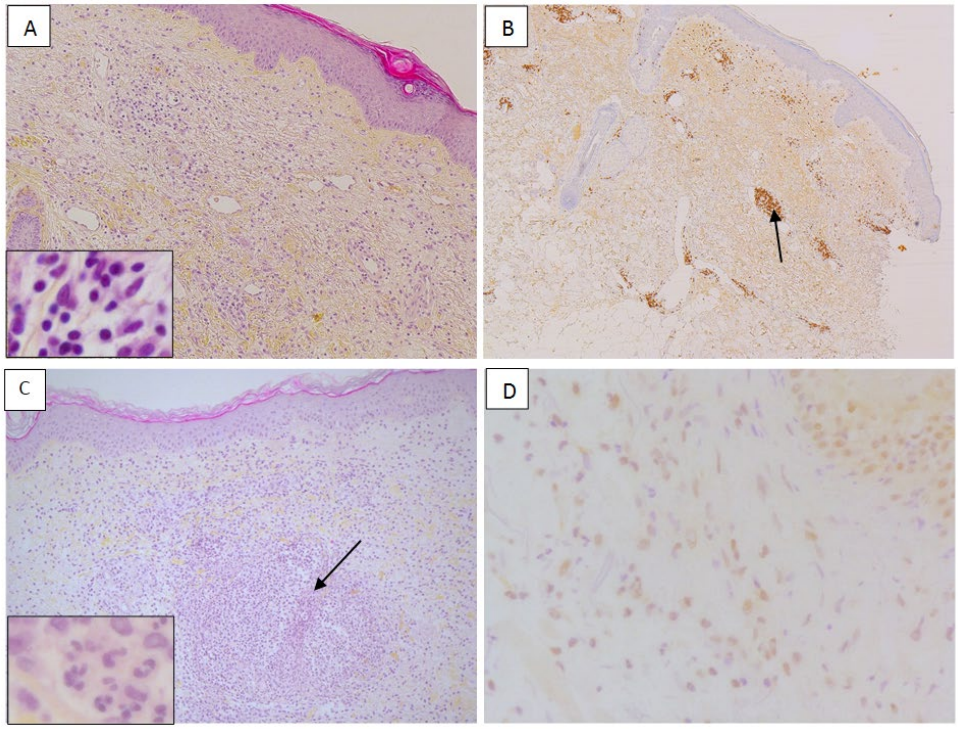


Figure 5