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TNFAIP3 haploinsufficiency is the cause of autoinflammatory manifestations in a patient with a deletion of 13Mb on chromosome 6

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ABSTRACT

There is scarce literature about autoinflammation in syndromic patients. We describe a patient who, in addition to psychomotor and growth delay, presented with fevers, neutrophilic dermatosis, and recurrent orogenital ulcers. Comparative Genomic Hybridization (CGH) array permitted to identify a 13.13Mb deletion on chromosome 6, encompassing 53 genes, and including TNFAIP3 gene (A20). A20 is a potent inhibitor of the NF-kB signalling pathway and restricts inflammation via its deubiquitinase activity. Western blotting and immunoprecipitation assays showed decreased A20 expression and increased phosphorylation of p65 and IkBa. Patient's cells displayed increased levels of total K63-linked ubiquitin and increased levels of ubiquitinated RIP and NEMO after stimulation with TNF. We describe the molecular characterization of an autoinflammatory disease due to a large chromosomal deletion and review the phenotypes of patients with A20 haploinsufficiency. CGH arrays should be the first diagnostic method for comprehensive analysis of patients with syndromic features and immune dysregulation.

1. Introduction

Syndromic immunodeficiencies are defined as those conditions where immunological abnormalities are part of a broad phenotype due to defective embryogenesis, metabolic disorders, chromosomal abnormalities or teratogenic disorders [1]. Some of these genetic disorders are well recognized syndromes, such as ataxia-telangiectasia or Wiskott-Aldrich syndrome, in which a well-defined and persistent immune deficiency presents together with non-immune organ dysfunctions. Moreover, other well-established genetic syndromes like Down or Turner syndrome are associated with a higher frequency of B and T-cell defects [2]. In the past ten years, thirteen cases, three patient series, and two families with chromosomal aberrations and immunological abnormalities have been described in the literature [3]. These studies

Abbreviations: ESID, European Society for Immunodeficiencies; ENT, Ear-nose-throat; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells; ALPS, Autoimmune lymphoproliferative syndrome; IPEX, Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome; FMF, Familial Mediterranean fever; TRAPS, TNF Receptor-Associated Periodic Syndrome; HLA, Human leukocyte antigen; NLRP3, NOD-like receptor family, pyrin domain containing 3; EBV, Epstein-Barr virus; MLPA, Multiplex ligation-dependent probe amplification

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illustrate the need of considering genomics structural variants in syndromic patients presenting with features of immunodeficiency and highlight the importance of carrying out immunological investigations in patients with chromosomal aberrations [2]. In an ESID survey published last year, a retrospective study was carried out to identify patients with immunological manifestations associated with chromosomal aberrations. From that study, it is remarkable that although recurrent ENT and airway infections were the most common clinical features in syndromic patients, many of the patients also presented with autoimmune diseases [3]. Association with autoinflammatory phenotypes has not been frequently reported. There is one patient with a homozygous deletion of 175kb on chromosome 2q13 affecting the *IL1RN* gene and a 28kb genomic deletion in a patient with deficiency of ADA2 (DADA2) [4,5]. Autoinflammatory diseases are characterized by seemingly unprovoked episodes of fever and organ specific inflammation in the absence of high-titer autoantibodies or antigen-specific T cells and they are caused by dysregulation of the innate immune system [6,7]. Genetic defects underlying these conditions are found in molecules involved in regulation of IL-1 signaling, NF-kB activation and/or type 1 IFN signaling [8]. To date, molecular characterization has been achieved in many patients presenting as familial cases with well-defined autoinflammatory diseases. However, a large number of both early and late-onset sporadic cases remain unexplained. It is widely accepted that many immunological diseases present as a continuum, with rare monogenic immune dysregulation diseases like ALPS or IPEX in one end of the spectrum, and rare monogenic autoinflammatory diseases like FMF or TRAPS on the other end [9]. However, some immunological diseases can be polygenic or influenced by modifying alleles giving rise to complex and overlapping clinical phenotypes [10]. This significant heterogeneity is exemplified when observing the genetic landscape of Behçet's disease (BD). Behçet's disease is a multisystemic inflammatory disease characterized by recurrent oral ulcers, ocular involvement, genital ulcers, and skin lesions that present with flares [11]. Despite the fact that most patients with BD are diagnosed during adulthood, a significant proportion of cases (4-26%) are diagnosed in childhood highlighting the possibility of causative monogenic disorders [12]. In this sense, apart from the genetic association with HLA-B*51 and additional independent associations to class I HLA regions, genome-wide association studies and next-generation sequencing have revealed the involvement of many other non-HLA related genes to the disease. Nowadays, the genetic landscape of Behçet's disease spans from common, low-penetrance variants to rare, high-penetrance mutations, like the ones observed in *TNFAIP3* (TNF-alpha induced protein 3) [9,13,14].

TNFAIP3 is a gene located on the chromosome 6q23.3 and it encodes for the A20 protein, which is involved in the negative regulation of NF-kB signalling through its dual action of ubiquitinase and de-ubiquitinase (DUB). A20 de-ubiquitinates the K63 Ub chains on key signalling molecules including the receptor interacting protein (RIP) and the NF-kB essential modulator (NEMO) and can ligate K-48 Ub chains to RIP and IκBα, tagging them for proteasomal degradation [15]. Apart from its involvement in NF-kB signalling, it has been recently discovered that A20 plays a critical role in preventing spontaneous NLRP3 inflammasome activation [16,17].

Genome wide association studies (GWAS) have associated many mostly non-coding SNPs with increased susceptibility to different autoimmune and inflammatory diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis, psoriasis, coeliac disease, type 1 diabetes, Crohn's disease, systemic sclerosis and coronary artery disease in type 2 diabetes. Because of the type and localization of the SNPs, it was suggested that the reduced A20 expression could be the factor conferring susceptibility to inflammation [18]. Direct evidence that reduced levels of A20 expression cause autoimmune or inflammatory disease was revealed with the observation that mice with either complete or cell-specific A20 deficiency develop spontaneous inflammation [18]. In 2016, Zhou et al. described a novel autoinflammatory disease in patients with early-onset systemic inflammation resembling BD due to haploinsufficiency of A20 (HA20) [19]. This first study described 5 families with a dominantly inherited disease due to heterozygous loss-of-function mutations in the *TNFAIP3* gene (for a more detailed clinical information, please refer to [20]). Soon after that first publication, nine families of the Japanese ancestry were reported [21–24]. Although early-onset BD-like phenotype is the most common presentation of the disease, *TNFAIP3* haploinsufficiency is not restricted to this condition, since it has been also reported in patients resembling autoimmune lymphoproliferative syndrome (ALPS) [23] or with complex autoimmunity without lymphoproliferation [25].

In this manuscript, we describe the first patient presenting with concomitant features of autoinflammatory disease, HA20, and other syndromic manifestations caused by a large genomic deletion on chromosome 6.

2. Materials and methods

2.1. Patient

Clinical data were obtained from the patient's medical chart. A written informed consent was obtained from the patient's parents for the studies here reported according to the procedures of the Institutional Ethical Review Board of Hospital Universitari Vall d'Hebron, Barcelona, Spain.

2.2. Targeted next-generation sequencing (TruSight One Sequencing Panel)

Genomic DNA was extracted from EDTA-containing whole blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Targeted gene sequencing was performed using the TruSight One sequencing panel on the Miseq platform (Illumina, San Diego, USA) according to the manufacturer's instructions. TruSight One is a commercial next-generation sequencing (NGS) panel for targeted genomic enrichment of 4813 genes having known association with clinical phenotypes. Data analysis was performed using the Variant Studio Software (Illumina).

2.3. Microarray-based comparative genomic hybridization (aCGH)

DNA copy-number variations were investigated with an Array Comparative Genomic Hybridization assay (aCGH) using an 8x60K G4827A CGH ISCA v2 array design (Agilent, USA), according to manufacturer recommendations on DNA extracted from peripheral blood with GENTRA Puregene Kit (Qiagen, Hilden, Germany). Data analysis was performed by Cytogenomics 2.1. software with ADM-2 algorithm and a minimum of 3 consecutive probes to detect an anomaly.

2.4. Methylation-Specific MLPA (MS-MLPA)

CpG methylation and copy number changes in the 6q24.2 region were investigated with MS-MLPA assay using an ME032-A1 probe mix according to manufacturer recommendations (MRC Holland, Amsterdam, The Netherlands).

2.5. Cell culture

Primary fibroblasts were obtained from skin explants in 25 cm2 culture flasks with Eagle's Minimal Essential Medium (MEM) supplemented with nonessential amino acids, 20% foetal calf serum (FCS), and antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin). The flasks were maintained at 37 °C in a cell incubator with 5% CO2 and a relative humidity of 95%. At 14 days the fibroblasts occupied most of the surface of the dish between the explants and were released by enzymatic digestion with trypsin and further subcultured in 25 cm2 plastic flasks (passage 1). The cultures were fed by changing the

medium twice weekly with 5 ml of 10% FCS medium. At confluence (23 days), the cells were trypsinized, resuspended in MEM containing 20% FCS and 10% DMSO, and stored in liquid nitrogen for further analyses.

2.6. Antibodies and expression plasmids

Antibodies specific for TNFAIP3 N-terminus (sc-166692), Actin (scx1615), RIP (sc-7881, scx133102), NEMO (scx56919, scx8330), Ub (sc-271289) are from Santa Cruz(Santa Cruz Biotechonlogy, Texas, USA); TNFAIP3 N-terminal specific (#5630), K63-linkage Polyubiquitin (#5621), Phospho-IKKα/β (#2697), IKKb (#8943), IκBα (#4814, #9242), Phospho-IκBα (#2859), PhosphoP65 (#3033), RIP (#3493), Phospho-p38 MAPK (#4511), Phospho-p44/42 MAPK (Erk1/2) (#4370), Phospho-SAPK/JNK (#4668), p44/42 MAPK (Erk1/2) (#4695), SAPK/JNK (#9252), p38 MAPK (#8690), HRP-linked anti-rabbit IgG (#7074), HRP-linked anti-mouse IgG (#7076) are from Cell Signalling (CST, Danvers, MA, USA); and recombinant human TNF-a (300x01A) is from Peprotech (Rocky Hill, NJ, USA).

2.7. Cell Cultures and stimulation, immunoprecipitation and immunoblotting

Skin fibroblast cells derived from *TNFAIP3*-deficient patient or normal donors were grown in Dulbecco's modified Eagle's medium (Life Technologies, Carlsbad, CA, USA) plus 10% foetal calf serum and 1x antibiotics (Life Technologies, Carlsbad, CA, USA). Recombinant human TNF-α (20 ng/ml) was used to stimulate fibroblast cells (about $5x10^6$ cells/treatment) from 10 min, 20 min to 1 hr. Whole cell lysis were prepared using ice-cold 1-cell lysis buffer (Cell Signalling, Danvers, MA, USA) supplemented with complete protease inhibitors. Immunoprecipitation and immunoblotting were conducted as described previously [26–28] with specific antibodies.

3. Results

3.1. Case report

The patient is a 12 year-old Spanish boy. There is no consanguinity or significant family history except type 1 Von Willebrand disease in the father.

The patient was born at 37 weeks of gestation with moderate intrauterine growth delay. During his first year of life, he presented with several upper respiratory infections, gastroenteritis and febrile episodes of unknown origin that required hospital admission. In the context of a clear growth and psychomotor delay at the age of 7 months, a karyotype was performed and chromosomal fragility studied showing no abnormalities. At the age of 12 mo and 36 mo he developed

Table 1
Main immunological findings in our patient.

	Patient's results (6y.o.)	Age-matched reference values[a]
Immunoglobulin levels		
IgA (mg/dL)	286	45-230
IgG (mg/dL)	1721	608-1574
IgM (mg/dL)	340	40-230
IgE (KU/L)	291	0-63
Auto-antibodies		
Anti-nuclear antibodies (IIF)	Positive Speckled (1:160)	
Anti-ENA antibodies (SS/B, SS/A, RNP, Scl70, Jo1)	Negative	
Lymphocyte subsets (cells/uL)		
Total lymphocyte	1500	1200-3400
T cell (CD3+) %/absolute number	72 (1080)	55-78 (700-4200)
CD3+CD4+ %/absolute number	23 (345)	27-53 (300-2000)
CD3+CD8+ %/absolute number	48 (720)	19-34 (300-1800)
CD4/CD8 ratio	0.48	0.9-2.6
B cell (CD19+) %/absolute number	13 (195)	10-31 (200-1600)
NK (CD16+56+) %/absolute number	15 (225)	4-26 (90-900)
Lymphocyte activation markers		
HLA-DR+ in CD4+ %	19	3-14
HLA-DR+ in CD8+ %	53	3-14
Functional in vitro tests		
Lymphocyte proliferation (by using anti-CD3 +/- IL-2)	Normal	
Respiratory burst test	Normal	
Median acute phase reactants median (min-max)		
ESR (mm/1h)	44.18 (20-101)	< 15
CRP (mg/dL)	11.36 (0.05-44.93)	< 0.5

Abbreviations: NTU: Nephelometric Turbidity Units ENA: Extractable nuclear antigens.

Age-specific paediatric reference ranges for immunoglobulins and complement proteins on the Optilite™ automated turbidimetric analyser. Marina Garcia-Prat*, Gemma Vila-Pijoan*, Susana Martos Gutierrez, Guadalupe Gala Yerga, Esther García Guantes, Mónica Martínez-Gallo, Andrea Martín-Nalda, Pere Soler-Palacín, Manuel Hernández-González. Journal of Clinical Laboratory Analysis. Accepted.

Extended 8 colour immunophenotype for the study of Primary Immunodeficiencies: Reference Values in Healthy Children. Marina Garcia-Prat, Daniel Alvarez, Aina Aguiló-Cucurull,Clara Franco-Jarava, Andrea Martin-Nalda, Roger Colobran, Manuel Hernandez Gonzalez, Pere Soler-Palacin, Mónica Martínez-Gallo. Submitted.

[a] Reference values obtained from.

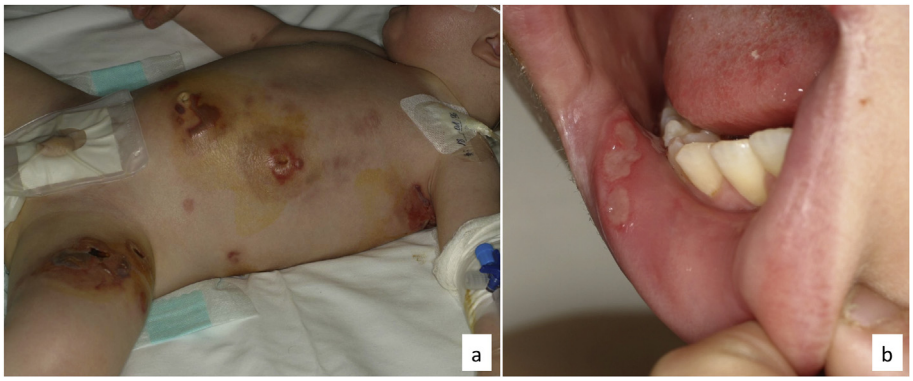

Fig. 1. Dermatological features in the patient with the deletion in chr6:134.387.945x147.518.246 region.
A. Purplish, confluent, infiltrated and painful plaques and nodules with tense serohemorrhagic blisters and necrosis. B. Recurrent episodes of painful ulcerated lesions on the oral mucosa with aphthous morphology.

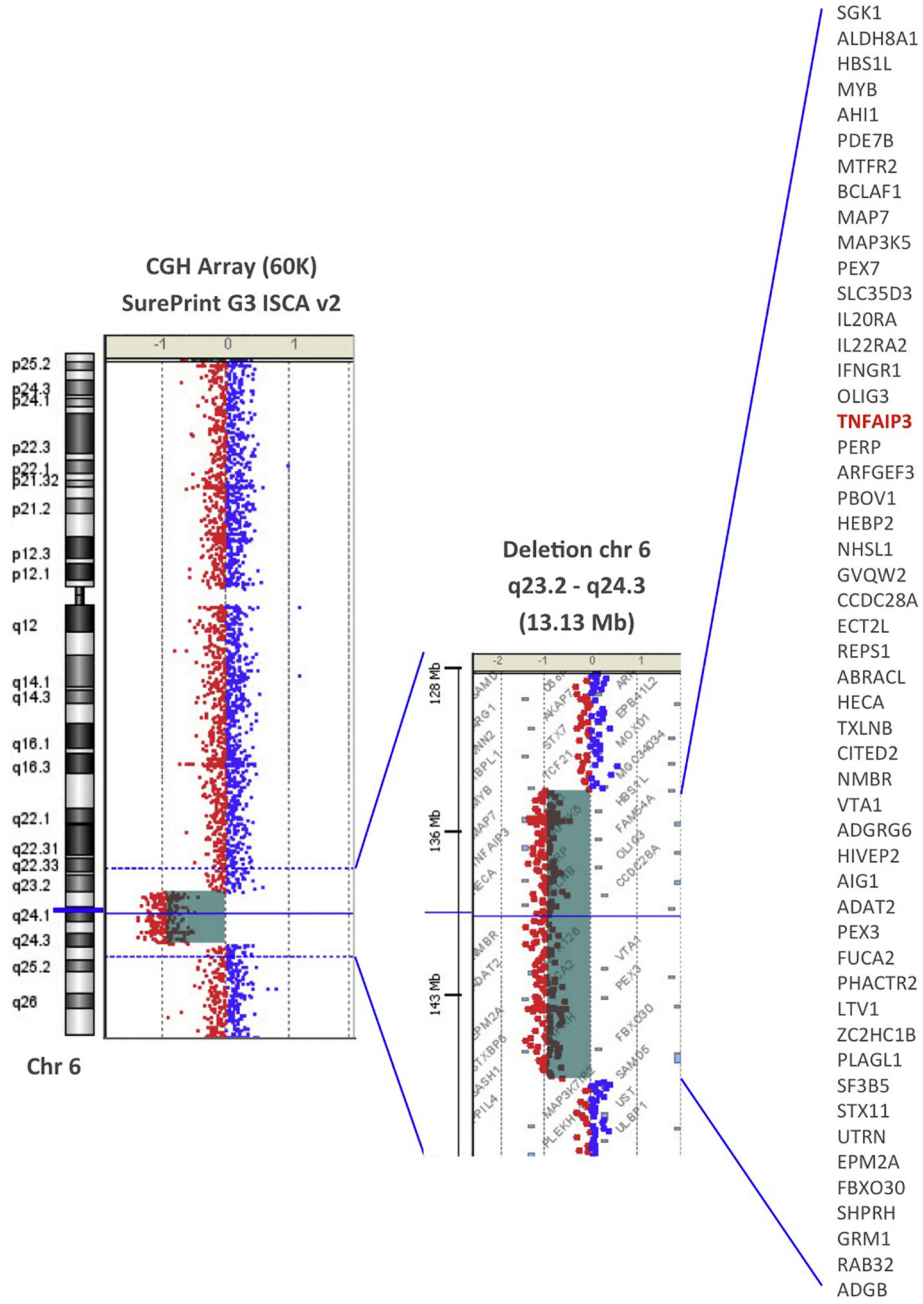

Fig. 2. Array CGH based identification of the 13.13 Mb deletion within the 6q23.2-q24.3 chromosomal region. The list of genes encompassed by this deletion is shown, including the *TNFAIP3* gene (in red).

neutrophilic dermatosis (see Fig. 1) with systemic involvement that required corticosteroid therapy. From the age of 5 y he presented with recurrent oral aphthae (see Fig. 1), diarrhoea and perianal ulcers always accompanied with increased acute phase reactants (for a detailed clinical description, please refer to Supplementary material).

His immunological profile revealed slight but persistent increased levels of polyclonal IgG, IgM and IgA (see Table 1), with a moderate increase in IgE levels. An inverted CD4/CD8 ratio was the only significant abnormality of the lymphocyte subpopulations. Cytotoxic NK cell activity was normal. The patient presented positive antinuclear antibodies with a speckled pattern at 1:160 titres but negative ENA specificities.

3.2. Genetic analysis

We conducted targeted next-generation sequencing using the TruSight One Sequencing Panel (Illumina) in the patient. This large panel, also known as clinical exome, includes 4813 genes with clinical relevance. Results showed no clear candidate variants that could explain the clinical phenotype of the patient. Due to the patient's cognitive and growth delay an aCGH was performed and we identified a deletion of 13.13 Mb on chromosome 6q23.2 (ISCN formula: arr [GRCh37] 6q23.2q24.3(134387945_147518246)x1) (see Fig. 2). Because of the aCGH results, we performed a methylation-specific multiplex ligation-dependent probe amplification (MLPA) v assay showing PLAGL1 gene (located in 6q24.2 chromosome band) hypermethylation. This gene is imprinted, with preferential expression of the paternal allele in many tissues and thus, deletion is assumed to be on the paternal

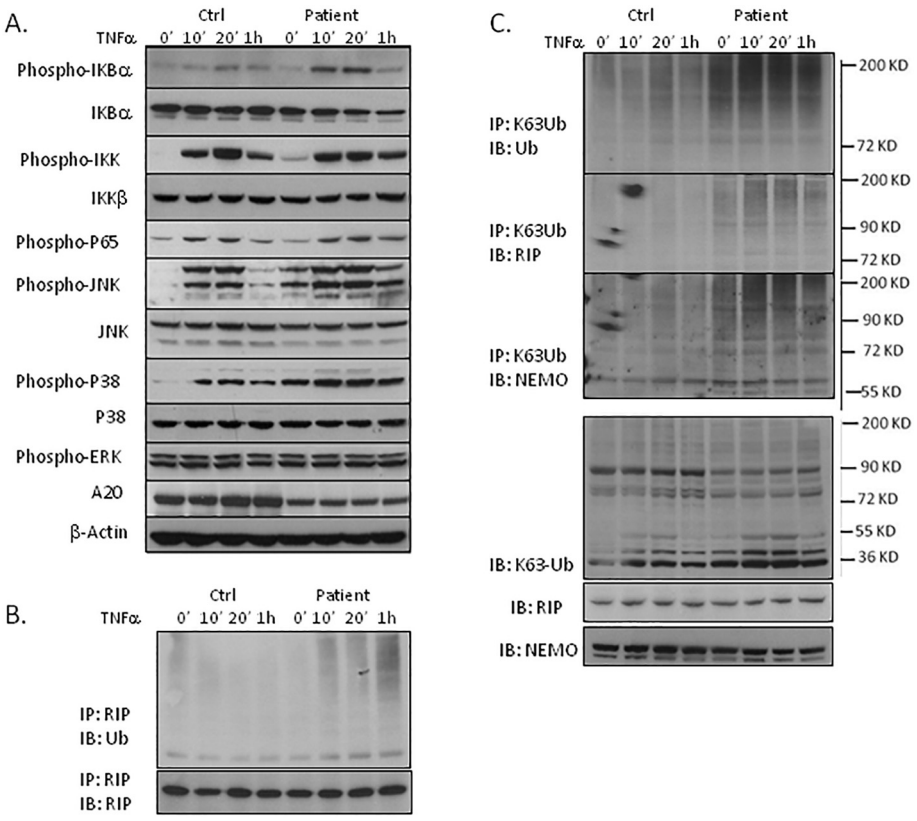

Fig. 3. Patient's cells display enhanced signaling in the NF-κB pathway.
(A) Skin fibroblasts from the TNFAIP3/A20-deficient patient or normal donor were stimulated with TNF-α (20 ng/ml). Immunoblots against phospho-IKKα/β, phospho-IkBα, phospho-JNK, phospho-P38, and A20 were conducted with whole cell lysates. TNF-stimulated TNFAIP3/A20 deficient cells display increased ubiquitination of RIP (B) and increased levels of K63 ubiquitinated NEMO and RIP (C).

chromosome. *In situ* fluorescence hybridization assays performed on both parents' samples confirmed that the deletion appeared *de novo* in the patient.

With the aim of identifying an explanation of the autoinflammatory phenotype, we performed a search of the 53 haploinsufficient genes. Among these genes (see Supplementary Table I), we identified six that could be linked to the immune system: *MAP3K5, IL20RA, IL22RA2, IFNGR1, TNFAIP3,* and *STX11*. Either because no disease-causing mutations had been previously reported in humans (*MAP3K5, IL20RA, IL22RA*) or due to an autosomal recessive inheritance pattern (*STX11*), the only genes left for consideration were *IFNGR1* and *TNFAIP3*. Autosomal dominant mutations in *IFNGR1* have been described in association with increased susceptibility to mycobacterial infections [29], which were absent in our patient. Therefore, *TNFAIP3* was the most likely candidate gene to explain the patient's autoinflammatory phenotype.

3.3. Functional analysis

Patient's fibroblasts showed a decreased A20 expression compared to control fibroblasts (see Fig. 3A bottom panel). We studied the activity of the NF-κB pathway in TNF-stimulated patients' fibroblasts. Sequential phosphorylation of IKKs and IκBα are essential steps in activation of the canonical NF-κB pathway. Patient-derived fibroblasts sustained higher levels of phosphorylated IKK and IκBα, showed increased phosphorylation of p65, and P38 and JNK MAP-kinases compared to healthy controls. These results are hallmarks of increased NF-κB signaling. Consistent with the haploinsufficiency of A20, patient's fibroblasts showed a marked defect in the de-ubiquitination of A20 target proteins: RIP and NEMO. Thus, monoallelic expression of A20 explains the inflammatory manifestations in this patient.

4. Discussion

We have identified a deletion of 13.13 Mb on chromosome 6 in a child presenting with skin, mucosal and gastrointestinal inflammation together with severe growth and psychomotor delay using a CGH array.

As it has been previously mentioned, conventional karyotyping showed no alterations, despite the size of the deletion is within the resolution of conventional karyotypes. The fact that the deletion is located in an interstitial chromosome region could probably be the reason why the deletion had not been detected in the first study. Since these situations are common in genetic diagnostic laboratories, the use of classical karyotypes has been substituted by CGH arrays.

This structural variant involves more than 50 genes and explains the patient's clinical phenotype including failure to thrive as well as the neurological impairment. Patients with similar genomic deletions show intellectual disabilities in varying degrees [30]. Deletion includes a differentially methylated region with two paternally expressed human genes, HYMAI and PLAGL1 [31] and paternal deletions involving 6q24.3 region are associated with intrauterine growth failure, early developmental delay, and characteristic facial appearance [32]. We have found only six constitutional cases characterized with array CGH and deletions involving TNFAIP3 gene. All deletions involve additional genes. The best characterized is that of Dutrannoy et al (2009) [30] which states that in cases with cytogenetic characterization (and thus uncertain breakpoints), chronic respiratory illness, apnea, or other abnormalities of respiratory control were described in 42% of these patients and also our patient had suffered from recurrent pulmonary infections during his infancy. However, clinical description is very poor and no laboratory tests are provided other than related to the characterization of the deletion. In the other five cases (249220, 251465, 257165, 259322 and 305663; Decipher database: https://decipher.sanger.ac.uk/) clinical information (very partial) is available only in two and there is not laboratory information other than array CGH results.

From an immunological point of view, the inflammatory phenotype of this patient is attributed to the heterozygous deletion of *TNFAIP3*, which leads to haploinsufficiency of the NF-kB regulatory protein A20. Although clinical history, CGH array results, and functional assays are

Table 2
Clinical features on patients presenting TNFAIP3 haploinsufficiency.

Family	Patient	Origin	Age of onset	*TNFAIP3* mutation	Fevers	Joint	Enlarged lymph nodes	Uveitis	Gut	Intestinal ulcers	Orogenital aphthae	Skin	Others	HLA-B51	Ref
1	P1	Eur-Canadian	20y	c.680T > A (p.Leu227*)		√					√		Recurrent URTI		19
	P2		10m		√	√		√	√		√				
	P3		15m			√		√	√		√		RTI		
2	P4	Eur-American	5y	c.671delT (p.Phe224Serfs*4)	√	√			√		√	√	Recurrent URTI		
	P5		6y		√	√					√	√			
	P6		10y		√	√		√	√	√	√	√	CNS vasculitis, ITP		
	P7‡		8y			√						√			
3	P8	Turkish	n.a.	c.811C > T (p.ARg271*)							√	√		+	
	P9		9m			√			√	√	√	√	Pericarditis in infancy	+	
4	P10	Eur-American	2m	c.1809delG (p.Thr604Argfs*93)	√				√	√	√		Recurrent RTI	−	
5	P11	Dutch	infancy	c.918C > G (p.Tyr306*)		√			√		√	√	Recurrent RTI		
	P12		4y						√		√		Rec. URTI/UTI	−	
6	P13	Japan	9y	c.728G > A (p.C243Y)	√					√	√	√	Nephrotic syndrome	−	22
	P14		8y							√	√				
	P15		8y		√						√				
	P16		10y								√				
	P17		12y								√				
7	P18	Japan	7m	c.1245_1248del (p.Gln415fs)	√		√		√			√	Kawasaki disease/ALPS		23
8		Japan	11y	c.252delC (p.Trp85GlyfsX11)	√	√		√	√		√	√	JIA/Behçet's disease	−	21
	P19														
	P20	Japan	9m			√			√		√		Recurrent hematemesis		
9	P21	UK	10y	c.1466_1467delTG (p.V489Afs*7)					√				DM-I, ILD, cytopenias		25
10	P22	Japan	2m	c.2088+5G > C (p.His636GlufsX55)	√	√						√	Aortic valve insufficiency	−	24
11	P23	Japan	6m	c.2209delC	√				√				FMF like		
	P24		5y	(p.Gln737SerfsX79)	√		√						Chron disease Hashimoto Thyroiditis		
	P25		Early childhood										Recurrent stomatitis, Hashimoto thyroiditis		
	P26		Early childhood										Recurrent stomatitis, Hashimoto thyroiditis		
	P27		1y		√								Stomatitis		
	P28		2m		√								Unidentified pneumonia		
12	P29	Japan	3y	c.1906+1G > A	√	√	√				√				
	P30		childhood	(p.Phe637GluX2)					√		√		IgA vasculitis		
13	P31	Japan	1y	c.1345delA	√						√		RBV-induced infectious mononucleosis		
	P32		childhood	(p.Asn449ThrfsX28)	√				√		√		Pneumocystis pneumonia		
14	P33	Japan	5d	c.1760_1770del11	√					√	√		periproctitis		
	P34		20y	(p.Ala588ValfsX80)	√						√		Grave's disease		
15	P35	Japan	1y	c.133G > T (p.Arg45X)	√		√			√	√				
16	P36	Turkey	29y	p.Pro268Leufs*19	v	√					√	√	Myalgia		20
	P37		15y								√				
	P38		13y								√				
17	P39	Unknown	1w	p.338X	√				√	√	√	√	CNS vasculitis, Pulmonary embolism		
18	40	Spain	2m	Chr6:134,387,945x147,518,246 del. (13.13Mb)	√		√		√		√	√	Kawasaki disease	−	⌗⌗

Abbreviation: URTI: Upper respiratory tract infection; RTI: respiratory tract infection; CNS: central nervous system; UTI: urinary tract infection; ITP: Idiopathic thrombocytopenic purpura; ALPS: autoimmune lymphoproliferative syndrome; JIA: Juvenile Idiopathic arthritis; DM-I: Diabetes Mellitus type I; ILD: interstitial lung disease, Abd: abdominal. * P7 corresponds to P5 in Aeschlimann [20] but belongs to the family previously described in Zhou [19] ⌗⌗ Current paper.

consistent with haploinsufficiency of A20, we did not perform whole exome sequencing, and therefore, we could not exclude the presence of other variants that may influence the patient's clinical phenotype. Table 2 summarizes the clinical features of the 40 patients with HA20 described in the literature. The information obtained relies on published data and some key details could have been omitted due to publishing space constraints. From the data obtained we can observe that despite of a mean age of onset of 6 years old, 12/40 patients present the first symptoms within their first year of life. Our patient first symptom, at the age of 2 months consisted of recurrent fever episodes. Although recurrent fever is observed in half of the patients with HA20 (23/40 patients), the most common clinical feature of this disease is the presence of recurrent oral and genital ulcers, which is described in 75% of the patients. This, together with the early age of onset, has lead to the denominate HA20 disease as autosomal dominant [22] or juvenile-onset Behçet's disease [21]. In addition, our patient also shared some clinical features (enlarged lymph nodes, coronary vasculitis) with the autoimmune lymphoproliferative syndrome-like patient described by Takagi et al [23]. Although our patient is the only one presenting with Sweet's syndrome, other forms of neutrophilic dermatosis (e.g. erythema nodosum) have been previously described. Noteworthy, gut manifestations, including inflammatory bowel disease, which was present in 17 of the 40 patients, has been observed to occur together with Sweet's syndrome [33].

Table 2 also reflects the genotype heterogeneity, variable phenotype expressivity and penetrance of HA20 disease. Almost all disease-associated mutations cause a premature stop codon which either form truncated mutant proteins or suffer nonsense-mediated decay (the exact effect of the different variants has not always been demonstrated). Genetic and functional analysis in a Japanese family diagnosed with dominantly inherited Behçet disease disclosed a heterozygous missense p.C243Y mutation in the OTU domain of A20/TNFAIP3. The ALPS-like Japanese patient carried a four-nucleotide deletion that also lead to a premature stop codon [23]. The type of mutation does not seem to correlate with the clinical phenotype and there is a significant phenotypic variability among the family members, suggesting a role for modifying alleles. To our knowledge the present study describes the first patient with a complete deletion of one of the *TNFAIP3* alleles, included in a larger chromosomal deletion on chromosome 6. The pathogenic effects derived from A20 haploinsufficiency have been demonstrated by Zhou et al., who showed that heterozygous loss of function *TNFAIP3* mutations are associated with a defect in removal of K63-linked ubiquitin from molecules involved in the regulation of the NF-kB signalling pathway and up-regulated transcription of NF-kB target pro-inflammatory cytokines IL-1β, TNF, IFNγ, IL-6 and IL-18. Moreover, since A20 is also a negative regulator of the NLRP3 inflammasome, haploinsufficiency of the protein leads to the constitutive activation of NLRP3. The results obtained from experiments in our patient's fibroblasts confirm that the genomic deletion encompassing *TNFAIP3* is the cause of the inflammatory phenotype in otherwise complex syndromic presentation.

Traditionally, corticosteroids have been the first line therapeutic option for autoinflammatory conditions but they are associated with potential severe adverse events derived from their prolonged use [34]. Therefore, cytokine blockers have become an eligible steroid-sparing option in these entities. Identifying the genetic defect underlying patients' autoinflammatory phenotype should permit a more adequate selection of first line therapeutic regimen. Since both NF-kB and NLRP3 inflammasome pathways are affected in the HA20 disease, the use of either IL-1 or TNF- inhibitors seem to be reasonable therapeutic options. To our knowledge, nine patients received steroids; seven HA20 patients have been treated with TNF inhibitors [19,21], one with an IL-1 inhibitor [19], and one with an IL-6 inhibitor [21]. Moreover, the case of a patient with HA20 that successfully underwent hematopoietic stem cell transplantation has been recently reported [25]. This patient had been previously treated with anti-TNF therapy, which resulted ineffective. This same treatment of TNF inhibitor, etanercept, has been recently initiated in our patient. However, the clinical response still needs to be properly assessed. To conclude, we want to highlight the importance of interdisciplinary team work to ensure an adequate management of patients presenting with syndromic and immunological features.

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