ADA2 deficiency (DADA2) as an unrecognised cause of early onset polyarteritis nodosa and stroke: a multicentre national study

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ABSTRACT

Objectives To analyse the prevalence of *CECR1* mutations in patients diagnosed with early onset livedo reticularis and/or haemorrhagic/ischaemic strokes in the context of inflammation or polyarteritis nodosa (PAN). Forty-eight patients from 43 families were included in the study.

Methods Direct sequencing of *CECR1* was performed by Sanger analysis. Adenosine deaminase 2 (ADA2) enzymatic activity was analysed in monocyte isolated from patients and healthy controls incubated with adenosine and with or without an ADA1 inhibitor.

Results Biallelic homozygous or compound heterozygous *CECR1* mutations were detected in 15/48 patients. A heterozygous disease-associated mutation (p.G47V) was observed in two affected brothers. The mean age of onset of the genetically positive patients was 24 months (6 months to 7 years). Ten patients displayed one or more cerebral strokes during their disease course. Low immunoglobulin levels were detected in six patients. Thalidomide and anti-TNF (tumour necrosis factor) blockers were the most effective drugs. Patients without *CECR1* mutations had a later age at disease onset, a lower prevalence of neurological and skin manifestations; one of these patients displayed all the clinical features of adenosine deaminase 2deficiency (DADA2) and a defective enzymatic activity suggesting the presence of a missed mutation or a synthesis defect.

Conclusions DADA2 accounts for paediatric patients diagnosed with PAN-like disease and strokes and might explain an unrecognised condition in patients followed by adult rheumatologist. Timely diagnosis and treatment with anti-TNF agents are crucial for the prevention of severe complications of the disease. Functional assay to measure ADA2 activity should complement genetic testing in patients with non-confirming genotypes.

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Accepted 13 April 2017

INTRODUCTION

The deficiency of adenosine deaminase 2 (DADA2) is a recently described autoinflammatory disease caused by loss-of-function homozygous or compound heterozygous mutations in *CECR1* (Cat Eye Syndrome Chromosome Region 1) gene.[1 2] DADA2 is characterised by an early onset vasculopathy with clinical and histopathological features of polyarteritis nodosa (PAN), associated with haemorrhagic and ischaemic strokes.[1–3] Hypogammaglobulinaemia with reduction of memory and terminally differentiated B cells and plasma cells may be present.[1 4] A severe clinical picture dominated by cytopenia and lymphoproliferation has been also described.[5 6] Even if the disease's onset is commonly in the paediatric age, some patients with adulthood onset have been described as well.[2]

ADA2 has a homology to the ADA1 protein, which is associated with a form of severe combined immunodeficiency. ADA1 and ADA2 have a key role in the regulation of the purinergic signalling pathway by converting adenosine to inosine and 2′-deoxyadenosine to 2′-deoxyinosine, respectively.[7] While ADA1 is ubiquitously expressed in all cell types, ADA2 is mostly expressed in monocytes and cells of myeloid lineage.[7]

ADA2 acts as a growth factor, playing a pivotal role in the development of endothelial and haematopoietic cells.[7 8] ADA2 also displays an autocrine activity and is able to induce monocyte proliferation and macrophage differentiation.[9] Monocytes of ADA2-deficient patients display a defect in the differentiation of M2 (anti-inflammatory) macrophages, which leads to the prevalence of M1 (proinflammatory) cells.[1]

Eighteen mutations of *CECR1* have been detected so far.[3] The p.G47R mutation has been detected in homozygous state in most patients of Georgian Jewish and Turkish ancestries.[1 2] The estimated carrier frequency of this mutation in the Georgian Jewish population is 10%.[2] The p.R169Q mutation is most frequently found in the Caucasian populations living in the Northern Europe where carriers might be up to two in 1000 individuals.[5 6 10]

In this study, we have analysed the prevalence of *CECR1* mutations among patients with a clinical picture characterised by early onset PAN, livedo

reticularis or stroke referred to the Italian Pediatric Rheumatology centres.

PATIENTS AND METHODS

Since February 2014, a national survey among the Italian centres of Pediatric Rheumatology was performed. Criteria of inclusion in the study were (1) early onset livedo reticularis associated with chronic or recurrent signs of systemic inflammation, (2) haemorrhagic/ischaemic stroke or signs of peripheral nervous system involvement associated with systemic inflammation, and/or (3) previous diagnosis with childhood onset PAN.[11] The study was approved by the Ethical Review Board of G. Gaslini Institute.

Genetic analysis

Molecular testing was performed on DNA samples extracted from peripheral blood lymphocytes by standard methods. All nine coding exons (from 2 to 10) of the *CECR1* gene (NM_001282228) were analysed by means of amplification followed by direct sequencing; the intronic regions were not analysed. Primers for PCR amplifications, amplicon lengths and PCR conditions are listed in the online supplementary material table S1. All the PCRs were performed as previously described.[12] Patients 1, 2 and 3 were screened by QZ and IA, as previously described.[1]

Cytokine profile assessment in monocytes

Following purification by peripheral blood mononuclear cells (PBMCs) adherence,[13] fresh 10% fetal calf serum (FCS) RPMI medium was added and monocytes were incubated at 37°C, 5% CO_2 for 6–18 hours in the presence/absence of zymosan (20 μg/mL) and Lipopolysaccharide (LPS) (1 μg/mL). Supernatants were collected at experimental time points (ie, 6 and 18 hours) and samples stored at −80°C. Six hours released tumour necrosis factor (TNF)-α and 18 hours secreted interleukin (IL)-1β and IL-6 were then quantified by ELISA assay.

ADA2 enzymatic activity

ADA2 plasma levels were detected with ELISA kit (eBioscience). ADA2 activity was assessed in primary monocytes. PBMCs were isolated through Ficoll-Paque and monocytes isolated by adherence, incubated for 1 hour in 24-well plate with RPMI 1% penicillin–streptomycin (Sigma Aldrich), 1% L-glutamine (EuroClone) in 5% CO_2 at 37°C. Monocytes were then cultured in PBS in the presence of exogenous adenosine (Sigma Aldrich), with or without the ADA1 inhibitor (erythro-9-(2-hydroxy-3-nonyl)adenine, EHNA, Sigma Aldrich). After 4 hours of incubation at 37°C and 5% of CO_2, supernatants were collected and the activity evaluated through the measurement of the adenosine-derived products (inosine, hypoxanthine) in high-performance liquid chromatography.

Statistical analysis

Comparison among genetically confirmed patients with DADA2 and mutation-negative patients was performed with χ^2 test. Non-parametric Mann-Whithney U test was used for the biological assays.

RESULTS

Molecular characterisation

From March 2014 to June 2016, we enrolled 48 patients, who fulfil the study inclusion criteria, from 43 families that were identified in eight Italian centres: 14 patients with early onset livedo reticularis associated with chronic or recurrent signs of systemic inflammation, 13 patients with haemorrhagic/ischaemic stroke or signs of peripheral nervous system involvement associated with systemic inflammation, 20 patients with a previous diagnosis with childhood onset PAN.

Homozygous or compound heterozygous *CECR1* mutations were found in 15 patients coming from 11 families. Four variants (G47A, G47R, P251L, Y435C) had already been associated with DADA2;[1 2] six (c.138_144delG, L249P, R312X, E328D, P344L, T360A) were novel mutations predicted to have deleterious effects on the protein function (table 1 and supplementary figures 1 and 2).

Consanguinity (third cousins) was reported in the parents of patient 3. Patients 13 and 14 were born from apparently non-consanguineous parents living in geographic isolation. The remaining 12 patients were born from unrelated parents. The living parents of five families were also analysed: all of them were heterozygous for one *CECR1* mutation (online supplementary figure 3).

A single disease-associated mutation (G47V) was observed as the sole genetic defect in two affected brothers and in their unaffected father and brother (table 1 and online supplementary figure 3).

A number of common single-nucleotide polymorphisms (L46L, N53N, H335R, Y453Y) were found both in patients carrying deleterious *CECR1* mutations and in the remaining 31 'genetically negative' patients.

Clinical presentation and disease course of genetically confirmed patients with DADA2

The main demographic features and the clinical manifestations at disease onset and during follow-up of the 15 genetically confirmed patients with DADA2 are summarised in table 1 (see also online supplementary material): two patients were in the group of early onset livedo reticularis associated with chronic or recurrent signs of systemic inflammation, 10 patients in the group of haemorrhagic/ischaemic stroke/peripheral nervous system involvement associated with systemic inflammation (seven of them received a histological diagnosis of PAN), two patients in the group of a previous diagnosis with childhood onset PAN. Patient 12 did not fulfil the inclusion criteria presenting a milder phenotype but was included in the study since his brother (patient 11) presented a typical phenotype.

The mean age at molecular analysis was 16.5 years (range 1–35 years); nine patients were children, six adults.

The clinical features observed during the disease course are reported in table 2 and figure 1, and discussed in detail in the online supplementary materials. The disease course was chronic or recurrent in nine and six patients, respectively (table 1).

Acute phase reactants were elevated in all but one patient. No patient displayed severe haematological manifestations, such as cytopenia. Low immunoglobulin levels, requiring substitutive treatment (IgG <500 mg/dL), were observed in three patients only; patient 12 displayed a reduced level of IgM. None of the patients displayed a clear history consistent with recurrent infections. Of note, patient 7's sister, who had the same clinical manifestations as the brother (early onset livedo reticularis, ischaemic stroke and inflammation) died at the age of 18 due to a septic shock secondary to an episode of pyelonephritis.

Clinical features of patients with an incomplete genotype and heterozygous family members

Patients 16 and 17 were heterozygous for the p.G47V variant and presented with a full picture of a DADA2-like disease.

Table 1 Clinical and demographic data of genetically confirmed and heterozygous patients with DADA2

Pt./sex	Age at onset/at diagnosis	Disease course	Fever	Skin manifestations	Stroke (number)	Peripheral nervous system involvement	Hypertension	Immunohaemato-logical symptoms	Other symptoms	Biopsy	*CECR1* mutations
Genetically confirmed patients											
1 M	2 years/14 years	Chronic	Yes	Livedo reticularis Subcutaneous nodules	Yes (2)	Peripheral neuropathy	Yes	HGG	Growth hormone deficiency, arthralgia	Polyarteritis nodosa (skin)	R312X E328D
2 M	9 months/7 years	Chronic	Yes	Livedo reticularis	Yes (1)	Peripheral neuropathy	Yes	HGG	Small bowel invagination	Polyarteritis nodosa (bowel)	R312X E328D
3 M	6 months/8 years	Recurrent → chronic	Yes	Livedo reticularis	Yes (2)	Peripheral paresis of the VII cranial nerve Neurosensory hearing loss	Yes	No	Myocarditis	Not done	T360A T360A
4 M	1 year/10 years	Recurrent → chronic	Yes	Livedo reticularis Subcutaneous nodules Ecchymotic lesions	No	No	No	Recurrent infection of upper airways	Diarrhoea	Polyarteritis nodosa (skin)	G47A P251L
5 F	3 years/12 years	Chronic	Yes	Livedo reticularis Urticarial rash	No	No	No	No	–	Leucocytoclastic vasculitis (skin)	c.138/144delG T360A
6 M	5 years/19 years	Chronic	No	Livedo reticularis Necrotic ulcers of extremities	No	No	Yes (with PRES)	No	Small bowel invagination	Polyarteritis nodosa (skin)	L249P T360A
7 M	8 months/24 years	Recurrent → Chronic	Yes	Livedo reticularis Purpuric lesions	Yes (1)	Peripheral paresis of the III cranial nerve Optic neuritis	Yes	No	Hepatomegaly, splenomegaly	Polyarteritis nodosa (skin)	G47R T360A
8 F	7 years/7.5 years	Chronic	Yes	Livedo reticularis	No	No	No	Recurrent infections of upper airways	Hepatomegaly	Not done	Y453C Y453C
9 F	12 months/17 years	Chronic	Yes	Livedo reticularis Erythematous skin rash Necrotic ulcers of extremities	Yes (3)	Peripheral neuropathy	Yes	No	–	Polyarteritis nodosa (skin)	T360A T360A
10 F	5 years/5 years	Chronic	Yes	Livedo reticularis Necrotic ulcers Subcutaneous nodules	Yes (1)	Peripheral neuropathy	Yes	No	Hepatomegaly, splenomegaly, arthralgia, arthritis	Polyarteritis nodosa (skin)	T360A T360A
11 M	12 months/12 months	Chronic	Yes	Livedo reticularis	Yes (1)	Neurosensory hearing loss	Yes (with PRES)	No	Generalised adenopathy, diarrhoea, oral aphthosis, arthralgia, arthritis	Not done	L249P P344L
12 M	5.5 years/6 years	Chronic → Recurrent	Yes	No	No	No	Yes	HGG (IgM)	Hepatomegaly, splenomegaly, arthralgia	Not done	L249P P344L
13 F	3 months/24 years	Recurrent	Yes	Livedo reticularis	Yes (2)	Peripheral paresis of cranial nerves	No	No	oral aphthosis	Polyarteritis nodosa (skin)	T360A T360A
14 M	6 years/22 years	Recurrent	No	No	Intracranial haemorrhage	No	No	No	No	Not done	T360A T360A
15 M	7 years/7 years	Chronic	Yes	Livedo reticularis psoriasis	Yes (1)	Peripheral neuropathy	Yes	HGG	Arthralgia	Leucocytoclastic vasculitis (skin)	Y453C Y453C

Continued

Table 1 Continued

Pt./sex	Age at onset/at diagnosis	Disease course	Fever	Skin manifestations	Stroke (number)	Peripheral nervous system involvement	Hypertension	Immunohaemato-logical symptoms	Other symptoms	Biopsy	*CECR1* mutations
Heterozygous patients											
16 F	1.5 years/10 years	Chronic	No	Livedo reticularis Subcutaneous nodules	Yes (3)	No	Yes	HGG recurrent infections of upper airways	Diarrhoea, abdominal pain, colic ulcerations	Leucocytoclastic vasculitis (skin), panarteritis nodosa (bowel)	G47V/WT
17 M	2 years/9 years	Chronic	No	Livedo reticularis Subcutaneous nodules Necrotic ulcers of extremities	No	Peripheral neuropathy	Yes	HGG	Diarrhoea, abdominal pain, arthralgia	–	G47V/WT

F, female; HGG, hypogammaglobulinaemia; M, male; PRES, posterior reversible encephalopathy syndrome; Pt., patient.

Both of them had an early disease onset (20 and 24 months) manifesting with livedo reticularis, subcutaneous nodules and necrotic ulcers of extremities in one of them (figure 1). Patient 16 suffered from multiple episodes of symptomatic ischaemic stroke. Hypogammaglobulinaemia was present in both siblings. Their father and the younger brother were heterozygous for the same mutation and asymptomatic. No mutation was detected in the asymptomatic mother (online supplementary figure 3). The second disease-causing mutation, a possible null allele inherited from the mother, could not be identified despite the analysis of the *CECR1* transcript in all the five members of the family (data not shown).

The study of the families trees of some genetically confirmed patients with DADA2 allowed the identification of other members presenting some DADA2-related clinical manifestations. The DNA from some of these individual was available for the analysis of *CECR1* (online supplementary figure 3). Several individuals from the family of patients 9 and 10, displayed manifestations consistent with an inflammatory vasculopathy (online supplementary figure 3). The mother presented at the age of 46 with systemic vasculitis (skin rash, low-grade fever) with good response to treatment with steroids. A few months later she presented with an episode of stroke. The father died of a cardiovascular attack. A third sister presented with severe systemic hypertension at the age of 20 years, associated with persistent arthralgia, responding to steroids. Both the son of this sister and patient 10's son presented Kawasaki disease at the age of 1 year, responding to treatment with immunoglobulins. With the exception of the father, whose DNA was not available, all these paucisymptomatic individuals were heterozygous for the T360A mutation (online supplementary figure 3). Patient 4's father, heterozygous for the P251L mutation, presented a myocardial infarction at the age of 40 years. Unfortunately, none of the above heterozygous patients were analysed from a functional point of view, so far.

The family history of patients 13 and 14 was significant for several family members who presented with cerebrovascular or cardiovascular events, in some cases associated with inflammatory manifestations (online supplementary figure 3); however, genetic testing for the other members is not available.

Clinical characteristics of patients with negative genetic test

Thirty-one patients fulfilling the inclusion criteria of the study tested negative for mutations in *CECR1*. Complete clinical data were available for 21 of these subjects and compared with 15 genetically confirmed patients with DADA2 (table 2). Genetically negative patients had a later disease onset, a lower prevalence of livedo reticularis and a higher prevalence of subcutaneous nodules (table 2). They also displayed a less frequent central nervous system (CNS) involvement: only three patients reported CNS manifestations (one with two ischaemic strokes, one with a single haemorrhagic stroke, one with encephalitis). Peripheral neuropathy (in three cases secondary to the use of thalidomide) was more common in genetically confirmed patients. No difference was observed in the incidence of hypogammaglobulinaemia (table 2).

Among *CECR1*-negative patients, a 6-year-old girl from Sardinia presented the whole clinical spectrum associated with DADA2. At the age of 3 months, she presented livedo reticularis, fever, elevation of acute phase reactants associated with severe myocarditis and hypertension. The skin biopsy was consistent with a PAN. Persistent hypogammaglobulinaemia was also detected. High doses of steroid with subsequent slow tapering were able to control the

Table 2 Comparison between *CECR1* genetically confirmed and *CECR1*-negative patients

	Genetically confirmed patients with DADA2 (n=15)	*CECR1* gene negative patients (n=21)	p Value
Mean age at onset (range)	2.9 years (3 months–7.5 years)	7 years (2 months–16 years)	0.001
Fever (%)	13 (86)	18 (85)	NS
Skin manifestations (%)	13 (86)	19 (90)	NS
Livedo reticularis	13 (86)	8 (38)	0.008
Subcutaneous nodules	3 (20)	15 (71)	0.004
Ulcerations of extremities	3 (20)	2 (9)	NS
Other	5 (33)	11 (52)	NS
Biopsy (%)	10 (66)	10 (47)	NS
Polyarteritis nodosa	8 (53)	8 (38)	NS
Leucocytoclastic vasculitis	2 (13)	1 (4)	NS
Other	0	2 (9)	NS
CNS involvement (%)	10 (66)	3 (14)	0.003
Stroke	9 (60)	2 (9)	0.004
Intracranial haemorrhage	1 (6)	0	NS
Other	0	1 (4)	NS
PNS involvement (%)	9 (60)	2 (16)	0.004
Peripheral neuropathy	5 (33)	2 (16)	NS
Cranial nerve paralysis	3 (20)	0	NS
Hearing loss	3 (20)	0	NS
Other	1 (6)	0	NS
Hypertension (%)	10 (66)	8 (38)	NS
Gastrointestinal manifestations (%)	5 (33)	9 (42)	NS
Diarrhoea	2 (13)	2 (9)	NS
Abdominal pain	0	8 (38)	NS
Bowel ischaemia	2 (13)	0	NS
Other	2 (13)	2 (9)	NS
Immunologic manifestations (%)	6 (40)	4 (21)	NS
Hypogammaglobulinaemia	4 (26)	2 (9)	NS
Recurrent infections	2 (13)	3 (14)	NS
Hepatomegaly (%)	4 (26)	8 (38)	NS
Splenomegaly (%)	3 (20)	6 (28)	NS
Generalised adenopathy (%)	1 (6)	1 (4)	NS
Articular manifestations (%)	5 (33)	13 (61)	NS
Arthralgia	5 (33)	13 (61)	NS
Arthritis	2 (13)	6 (28)	NS

CNS, central nervous system; PNS, peripheral nervous system.

inflammatory manifestations; however, two episodes of stroke were observed when steroid was withdrawn.

ADA2 enzymatic activity

ADA2 activity in plasma samples was significantly lower in patients with DADA2 compared with age-matched healthy controls (online supplementary figure 4).

We specifically analysed the ADA2 activity of circulating monocytes through the evaluation of inosine and hypoxanthine concentrations in the supernatant of adenosine stimulated cells in the presence or in the absence of an ADA1 inhibitor. Patients with DADA2[1 2 4] showed a lower enzymatic activity, as demonstrated by the absence of inosine in patient cells supernatant, compared with seven healthy subjects. Notably, patient 4, presenting with a mild phenotype, displayed a minimal residual enzymatic activity (figure 2A). As expected, ADA1 activity was normal in all the subgroups analysed (data not shown). Two genetically negative patients with a history of cutaneous PAN and the absence of strokes displayed a normal ADA2 activity (Ctr1 and Ctr2, figure 2). Conversely, the Sardinian patient (Ctr3), presenting with a full-blown DADA2 phenotype but negative for *CECR1* mutations, displayed a complete lack of ADA2 activity (figure 2A), despite plasmatic levels of ADA2 comparable to those of the healthy donors (data not shown), suggesting that this patient likely carries atypical mutation(s) in *CECR1* that was not detected by standard sequencing.

Both clinically affected heterozygous patients (16 and 17) displayed a complete absence of enzymatic activity, similar to patients with biallelic mutations. Conversely, the two heterozygous asymptomatic family members (the father and the brother), as well as the mother, displayed a preserved enzymatic activity (figure 2B).

Long-term response to treatment in patients with DADA2

The treatments applied to patients with DADA2 are reported in table 3 (see also supplementary material). The response to treatment was considered (i) complete, in case of persistent control of inflammatory parameters with no disease flares or complications in the absence of any steroid treatment; (ii) partial, in case of a good control of disease activity with sporadic relapses and need of steroid on demand or increased steroid dosage and (iii) poor, in case of little or absent response with persistence of systemic flares and/or complications (table 3). All patients showed a partial response to NSAID and complete response to high dose of steroids. However, all patients with a chronic disease course relapsed following steroid tapering. The most severe manifestations of disease (ie, cerebral strokes and intestinal invagination) occurred at the time of steroid tapering or steroid withdrawal.

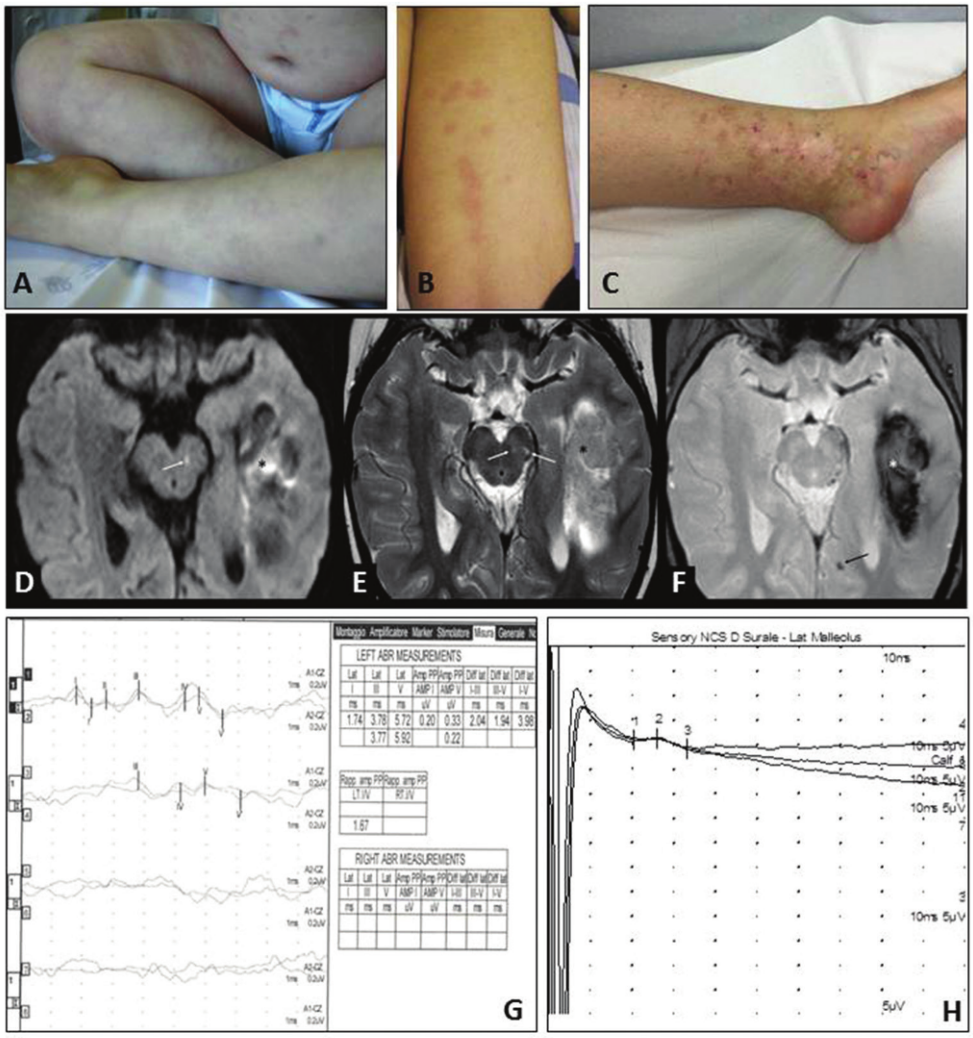

Figure 1 Clinical features in patients with adenosine deaminase 2 deficiency. (A) Livedo reticularis in patient 1. (B) Painful subcutaneous nodules in patient 4. (C) Scars after a deep necrotic ulcer at right lower limb in one heterozygous patient (16). (D–F) Brain MRI performed in patient 1 at 6 years of age before anti-TNF treatment. Axial T2* (D), diffusion (DWI) (E), T2-weighted (F) images show a large acute haemorrhagic infarct in the left temporal lobe (asterisks) associated with an acute small asymptomatic ischaemic infarct of the midbrain (E, arrows) and multiple small chronic lacunar infarcts at the level of the left cerebral peduncle (arrow, F). (G) Identification of complete acute hearing loss in patient 3 with auditory brainstem response (ABR). (H) Reduced amplitude of the sensitive action potential of the sural nerve following antidromic stimulation (mild absonal injury).

Immunosuppressive therapies (azathioprine, ciclosporin, cyclophosphamide, methotrexate, mycophenolate mofetil) were generally associated with a poor or partial response (table 3 and online supplementary material). Patient 5 had a poor response to treatment with an IL-1 receptor antagonist.

Interestingly, treatment with thalidomide (mean dose: 2 mg/kg/daily, maximum 50 mg/day) gave better results in seven patients (online supplementary material) with a complete response achieved in six patients. In three patients, the drug was withdrawn after 20 months, 25 months and 5 years due to neurological toxicity.

Ten patients received anti-TNF treatment with a complete remission in nine of them (table 3); only one patient was still steroid dependent. The median duration of treatment with etanercept in the 10 treated patients is now 3.9 years (range 0.9–13 years). No severe infections or other complications have been reported. The same good response was also observed in the heterozygous patients 16 and 17 (table 3).

We analysed the pattern of cytokine production from isolated monocytes in a patient with DADA2 with active disease (Patient 3) and three patients in remission (6, 7 and 9). LPS-stimulated monocytes from the patients with DADA2 with inactive disease displayed higher secretion of TNF-α compared with age-matched healthy donors (supplementary figure 5A). The TNF secretion was substantially higher in monocytes from patient 3 (supplementary figure 5B), with a clear downmodulation 1 month after the beginning of anti-TNF treatment (supplementary figure 4A).

DISCUSSION

The present paper describes the largest series of European Caucasian patients affected with DADA2. In this cohort, we identified six novel disease-causing mutations. We observed a variability of the clinical phenotypes associated with DADA2, and we describe a small number of patients presenting with typical clinical manifestations of DADA2 (vasculopathy, stroke, hypogammaglobulinaemia) and non-confirming or negative genetic analysis. We developed a novel enzymatic assay to analyse the ADA2 activity of circulating monocytes that was able to distinguish affected patients from healthy controls and healthy heterozygous carriers.

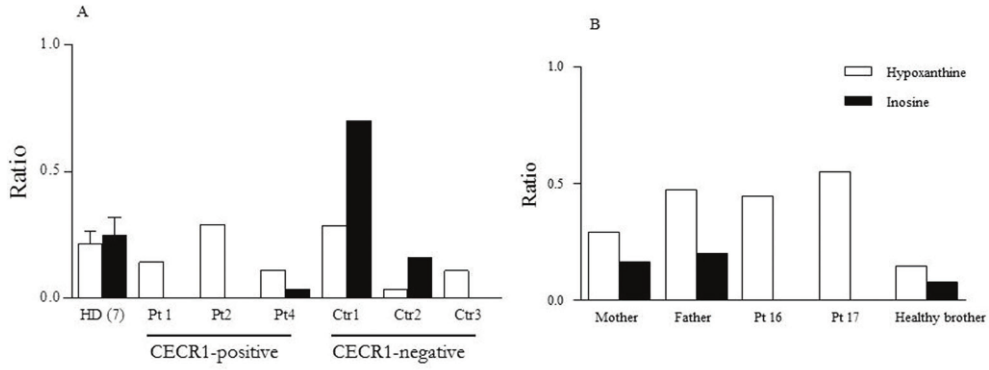

Figure 2 Adenosine deaminase 2 (ADA2) enzymatic activity was assessed in monocytes of patients with different ADA2 mutations compared with age-matched healthy controls. Inosine (black column) and hypoxanthine (white column), derivative products of the metabolism of adenosine mediated by ADA2, were measured in the monocyte supernatant after 4 hours of incubation with adenosine 15 µM and ADA1 inhibitor 30 µM. Values refer to the amount of adenosine present in the supernatants. (A) ADA2 enzymatic activity in patients 1, 2 and 4 in age-matched healthy donors (HD), in genetically negative patients with PAN (Ctr1 and Ctr2) and in the genetically negative Sardinian patient carrying a complete DADA2-like phenotype (Ctr3, see also text). (B) The complete loss of ADA2 enzymatic activity in the two heterozygous patients (16 and 17) with a typical DADA2-like phenotype and the normal activity in the two healthy family members carrying the same mutation and in the wild-type mother (see also text).

Our study confirms that treatment with anti-TNF therapies has a sustained beneficial effect in patients with DADA2, and we showed the first time that the treatment with thalidomide maybe an effective and less expensive alternative therapeutic strategy.

Our study shows a rather high prevalence of *CECR1* mutations in the Italian paediatric population diagnosed with a PAN-like vasculopathy and/or presenting with a history of strokes associated and inflammatory disease. The screening for *CECR1* mutations should be performed in adult patients with a similar phenotype. An early onset symptom, livedoid rash and a history of cerebral stroke were associated with a higher likelihood to identify *CECR1* mutations. Nevertheless, a milder disease course characterised by sole manifestations of a cutaneous PAN disease can be also present in *CECR1* mutation-positive patients (patient 4).

Despite this study covers most of the Italian paediatric patients with PAN vasculitis or stroke associated to inflammatory vasculopathy, it is conceivable that patients with a prevalence of haematological abnormalities may have been missed. The same could also be for patients with an adult onset or with a milder phenotype. Moreover, no data are so far available on the prevalence of *CECR1* mutations in the general Italian population.

Among the *CECR1* mutation-positive patients, a number of unreported mutations, mainly located in the catalytic domain of the protein, thus likely to disrupt the enzymatic activity, have been identified. Some of them, such as the T360A variant, appear to be rather specific for the Italian population, suggesting a possible founder effect. In three patients with the clinical and enzymatic phenotype consistent with DADA2, we identified either one (heterozygous) or none mutations in *CECR1*. Although our preliminary search for the putative second mutation did not produce results, it suggests that mutations affecting regulatory non-coding sequences or genomic deletions likely account for atypical mutations in this gene. This search requires a different set of analysis.

We observed the presence of clinical manifestations possibly associated with DADA2 in a number of family members who are heterozygous carriers. This finding could support the hypothesis of a possible digenic or polygenic inheritance, as recently observed in other autoinflammatory conditions.[14] Whole exome sequencing approach is currently ongoing in the Sardinian patient presenting with a complete clinical and functional DADA2 phenotype, with the aim to identify other genes that may affect the ADA2 enzymatic activity. Another possibility is that this patient carries either genomic deletion(s), intronic mutation(s) and/or regulatory mutation(s).

Our study describes a wide phenotypic variability and diseases expressivity in patients with DADA2. [1 2 5 6 15–18] The clinical course can be chronic or characterised by recurrent flares of systemic inflammation. In patients with a recurrent disease course, the most severe clinical manifestations (ie, strokes or other vascular accidents) were mainly observed during the inflammatory flares, thus suggesting the need for a continuous treatment in all patients. As previously reported, neurological manifestations of central and peripheral nervous system represent the most severe clinical features leading to permanent damage and/or functional dysfunction.[1 2] Acute and permanent unilateral hearing loss has been experienced during disease inflammatory flares in two patients of the present series and represents an additional complication of the disease. Myocarditis was the clinical manifestation at disease onset of patient 3 and was also reported in the Sardinian patient negative for *CECR1* mutations. This finding has anecdotally been associated with polyarteritis in children.[19]

Though severe cytopenia has been described in the spectrum of DADA2 associated phenotypes,[20] none of our patients displayed any haematological features. A minority of our patients displayed a hypogammaglobulinaemia with a history of recurrent infections, resembling clinical features of patients followed for common variable immunodeficiency (CVID).[21] This finding is likely due to the selection of our patients from the paediatric rheumatology community and should not underestimate the multifaceted phenotype of patients with DADA2, some of whom may display major haematological and/or immunological features of the disease.

In the present study, we confirm the dramatic and persistent efficacy of treatment with anti-TNF therapies that completely controlled the inflammatory manifestations preventing the occurrence of vascular events in all treated patients without severe complications.[2] Circulating TNF-α levels in patients with DADA2 are rather variable.[2 4] However, a clear expression of TNF was found in the inflammatory infiltrate of affected tissues.[2] In the present study, we had the opportunity to analyse the pattern of TNF production in LPS-stimulated monocytes

Table 3 Response to treatment in genetically confirmed and heterozygous patients with DADA2.

Pt	NSAIDs	Steroids*	Thalidomide	AZT	CTX	MMP	CyA	MTX	Anakinra	Adalimumab	Infliximab	Etanercept
1	Poor	Complete*	Complete	–	Poor	–	–		–	–	–	Complete
2	Poor	Complete*	Complete	–	–	–	–		–	–	–	Complete
3	Poor	Complete	–	–	–	–	–		–	–	–	Complete
4	–	Complete	–	–	–	–	–		–	–	–	Complete
5	Poor	Complete*	Partial	–	–	–	Poor	Complete (+adalimumab)	Poor	Complete (+MTX)	–	Complete
6	–	Partial	–	–	Poor	Complete	–		–	–	Complete	Complete
7	–	Complete*		Poor	–	Partial	–		–	–	–	–
8	–	Complete	–	–	–	–	–		–	–	–	Complete
9	–	Complete*	Complete	Poor	Complete (with low doses steroid)	poor	–		–	–	–	–
10	–	Complete*	Complete	Poor	–	–	–	Poor	–	–	–	–
11	–	Partial	–	–	–	–	–	–	–	–	–	Complete
12	–	Complete	–	–	–	–	–	–	–	–	–	Complete
13	–	Complete	Complete	–	–	–	–	Poor	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–
15	Poor	Complete*	Complete	Poor	Poor	–	Poor	–	–	–	–	Partial
16	–	Complete*	–	–	Poor	Partial	–	–	–	–	–	Complete
17	–	Complete*	–	Poor	–	Partial	–	–	–	–	–	Complete

*Only for doses higher than 1 mg/kg daily, with several relapses during tapering.

Complete, Persistent control of inflammatory parameters with no disease's flares or complications in the absence of any steroid treatment; *partial*, good control of disease activity with sporadic relapses and need of steroid on demand or increased steroid dosage; *poor*, little or absent response with persistence of systemic flares and/or complication.

NSAID, Non steroidal anti.inflammatory drugs; AZT, azathioprine; CTX, cyclophosphamide; CyA, ciclosporin A; MMP, mycophenolate mofetil; MTX, methotrexate.

of a patient with DADA2 with active disease and following the anti-TNF treatment.

Interestingly enough, the same persistent therapeutic effect obtained with anti-TNF agents has been observed in majority of the patients treated with thalidomide, which exerts a potent and specific anti-TNF effect.[22 23] Despite the possible toxic effects on peripheral nervous system, this drug has the advantage of a lower cost and could be considered in developing countries in which the use of biological treatments is economically limited.

Our experience suggests that the anti-TNF treatment should be initiated at the moment of the diagnosis, even in patients who do not have two identified mutations in *CECR1*. In these patients, functional assay should be used to confirm the lack of ADA2 activity. Although several reports showed that haematopoietic stem cell transplantation is a potential curative therapeutic strategy for patients with DADA2,[5] our study supports the use of anti-TNF treatment as a first choice intervention, at least in patients with a prevalent inflammatory phenotype. In our study, only one patient (patient 9) withdrew the effective treatment due to persistent complete well-being with a subsequent severe disease flare (see online supplementary material). We therefore suggest not to discontinue the effective treatment in patients with a clear enzymatic defect.

In conclusion, DADA2 may account for a number of patients with PAN-like disease, neurological manifestations and systemic inflammation in children and might represent an unrecognised condition in adult patients followed by adult rheumatologists. A timely diagnosis and a prompt treatment with anti-TNF agents are crucial for the prevention of severe complications of the disease, even in patients with non-conclusive *CECR1* genotype.

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Acknowledgements We would like to thank Drs E Cortis, L Breda and B Lattanzi who provided genetically negative patients, Professor M P Sormani for her assistance in the statistical analysis and Dr P Lanteri for the functional neurological studies. The study is partially supported by Ricerca Corrente Ministeriale of the Italian Ministry of Health.

Contributors RC, MG: coordination of the study, interpretation of the results, elaboration of the manuscript. FP, AO, FS: functional immunological studies. AG, QZ: genetic analysis. MS, CG: neuroimaging. AS, GD: biochemical studies. AI, MA, GC, FM, PP, AT, SM, CM, RG, RAP, FF, FG, AMo, MP: clinical characterisation of patients, interpretation of the results, critical reading of the manuscript. AMa, AR, IA, IC: interpretation of the results and critical reading of the manuscript.

Competing interests None declared.

Patient consent Patient or guardian according to the age.

Ethics approval G. Gaslini IRCCS Ethics Board.

Provenance and peer review Not commissioned; externally peer reviewed.

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Correction: *ADA2 deficiency (DADA2) as an unrecognised cause of early onset polyarteritis nodosa and stroke: a multicentre national study*

Caorsi R, Penco F, Grossi A, *et al*. ADA2 deficiency (DADA2) as an unrecognised cause of early onset polyarteritis nodosa and stroke: a multicentre national study. *Ann of Rheum Dis* 2017;

The author's name Romina Gallizi is incorrect and should be Romina Gallizzi.