Deficiency of adenosine deaminase type 2 (DADA2): a description of phenotype and genotype in 15 cases.

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ABSTRACT

Objectives: To describe the clinical features, genotype, and treatment of a series of subjects with confirmed Deficiency of Adenosine Deaminase 2 (DADA2) referred to Great Ormond Street Hospital NHS Foundation Trust (GOSH), London.

Methods All symptomatic subjects were referred for genetic testing for suspected DADA2; relatives of index cases were also screened. The demographic, clinical, laboratory characteristics and treatments were recorded. Genetic analyses performed included whole-exome sequencing in 4 patients; and Sanger sequencing of Cat Eye Syndrome Chromosome Region Candidate 1 (*CECR1)* in all subjects. ADA2 enzyme activity assay, and quantitative PCR of *CECR1* mRNA were also performed.

Results We identified 15 subjects with DADA2; 5 subjects were asymptomatic (relatives of index cases; age 5-42 years). Homozygous or compound heterozygous mutations in *CECR1* were identified in all subjects. The phenotypic manifestations of the symptomatic DADA2 patients included livedo racemosa (73.3%); neurologic involvement (53.3%); and immune deficiency (46.6%). *CECR1* mRNA expression in 8 subjects, including five pre-symptomatic subjects was significantly lower than healthy controls (p=0.0016). DADA2 subjects (with or without symptoms) also had lower ADA2 enzyme activity compared to healthy paediatric controls (p<0.0001), and sporadic cases of childhood polyarteritis nodosa without *CECR1* mutation (p=0.0108). Nine/10 symptomatic patients required anti-TNF-α therapy.

Conclusion The clinical severity of DADA2 ranged from limited cutaneous to severe multi-systemic vasculitis; one third (5/15) of our cases were currently asymptomatic, and required close monitoring. We recommend *CECR1* screening for: unaffected siblings of index cases; cases of familial vasculitis; and patients with polyarteritis nodosa recalcitrant to standard treatment.

INTRODUCTION

Loss of function mutations in the Cat Eye Syndrome Chromosome Region Candidate 1 (*CECR1*) gene cause Deficiency of Adenosine Deaminase 2 (DADA2) in patients with vasculopathy and clinical features compatible with polyarteritis nodosa (PAN) (1,2), a necrotizing medium vessel vasculitis (3). Clinical features of DADA2 frequently described in these first reports were positive family history, livedo racemosa, early-onset stroke, peripheral nervous system involvement, and immunodeficiency in some cases (1,2). The true breadth of the phenotype of DADA2 remains to be fully established. Another increasingly controversial issue regards the management of pre-symptomatic individuals with DADA2 detected as a result of family screening following identification of an index case. The purpose of this study was therefore to describe the clinical features, genotype, and treatment of a case series of subjects with confirmed DADA2 referred to a tertiary vasculitis referral centre, Great Ormond Street Hospital NHS Foundation Trust (GOSH), United Kingdom.

PATIENTS AND METHODS

Patients and controls

This study was approved by the Bloomsbury research ethics committee, London (REC reference 08/H0713/82); all subjects studied prior to publication of the initial reports of DADA2 provided fully-informed written consent to participate, and informed assent where appropriate. Following publication of the original

DADA2 reports, some subjects had *CECR1* screening as part of routine clinical care. Inclusion criteria were: 1. children and their relatives referred for genetic testing for suspected DADA2; and 2. availability of DNA for study purposes. Data recorded at the time of diagnosis were: sex, age, ethnicity, organ involvement, the serum C-reactive protein (CRP) level, histopathologic and arteriographic findings, and treatment.

We also performed functional analyses on samples from patients and their relatives whenever possible. Healthy reference ranges for adenosine deaminase 2 (ADA2) enzyme activity (see methods below) were derived from left over samples (n=22) from healthy paediatric patients for development of diagnostic tests for primary immunodeficiency (REC reference 06/Q0508/16). Reference ranges for healthy adults were derived from samples from healthy volunteer adult donors working in our laboratory (n=20), with consent. Sporadic childhood PAN (cPAN) patients without *CECR1* mutations served as disease controls for ADA2 enzyme activity. In addition, *CECR1* mRNA expression was evaluated in 7 paediatric and 1 adult patient with DADA2 (Table 1); 2 healthy child controls (different individuals from the controls used for ADA2 enzyme activity, with consent); and 4 healthy adults (who also had ADA2 enzyme activity assessed).

Genetic analyses

Whole-exome sequencing (WES) was performed in four patients where there was a strong clinical suspicion (familial cases; and/or onset in infancy) of a

monogenic cause for their vasculitis prior to the publication of the original descriptions of DADA2 (1,2). Other symptomatic cases were detected by Sanger sequencing of *CECR1* in patients with cPAN resistant to standard treatment. Confirmation by Sanger sequencing of *CECR1* was performed in all subjects, including those who initially underwent WES. Homozygosity mapping was also performed in one patient from consanguineous parents (family A). Library preparation for WES was completed using the Illumina Nextera Rapid Capture Exome Library Preparation Kit. WES was completed using the Illumina HiSeq 1000 and Illumina NextSeq 500 platform. Sanger sequencing was performed using the Applied Biosystems 3730 DNA Analyzer; see Supplemental Table S-1.

Quantitative PCR

RNA was obtained from peripheral blood samples collected in PAXgene blood RNA tubes (Qiagen). RNA isolation was performed using the PAXgene blood RNA kit (Qiagen). Complementary DNA synthesis was performed using High-Capacity RNA-to-cDNA kit (Lifetechnologies). Quantitiative PCR (qPCR) was performed using Power SYBR green PCR mastermix (Lifetechnologies) with specific primers for *CECR1* (QIAGEN: QT01667862) to analyse gene expression. β-actin was used as a reference gene. The qPCR was performed on the real time PCR system (Rotor-gene 6000). Analysis was done on the Rotor-gene 6000 Series software version 1.7, using the standard curve method for the quantification. Samples from each case were tested once.

ADA2 enzyme activity assay

Adenosine deaminase assay kit (Diazyme) was used to quantify ADA2 enzyme activity, as per manufacturer instructions (see supplemental methods). 100nM of erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), an specific adenosine deaminase 1 (ADA1) inhibitor, was added during the assay. Light absorbance at wavelength 540nm as a measure of ADA2 activity was assessed using a Fluorstar Optima spectrophometer. Samples from each case were tested once; the intra-test coefficient of variation (CV) for this assay is < 4.5%, and inter-test CV is < 5% (manufacturer's product sheet).

Statistical Analysis

Continuous variables were summarized as median and range. Categorical variables were presented as percentages and frequencies. Comparison of ADA2 enzyme activity and mRNA expression were performed using the Mann-Whitney U test. A p-value (two-tailed) of <0.05 was considered significant. All statistical tests were performed using GraphPad Prism version 5.

RESULTS

Demographic and clinical features of DADA2 patients

We identified 15 subjects with DADA2, confirmed with Sanger sequencing. Of these 15 subjects, 13 were seen at GOSH; 2 patients (siblings) were seen at

Queen Silvia's Children Hospital, Sweden. The clinical features of all the genetically confirmed DADA2 subjects are shown in Table 1. Five of the 15 subjects were asymptomatic (including patient C2, Table 1, who was asymptomatic, but had mild livedo racemosa detected on a screening clinical examination), and were relatives of index cases.

A family history of symptoms compatible with DADA2 was positive in 3 families. Two patients included in this series were born to related Iraqi/Iranian patients (family A); 2 patients were born to unrelated Indian parents (family B); and 2 patients were born to unrelated Caucasian Swedish parents (family G). In addition, regarding family G, the mother was noted to have a bicuspid aortic valve, and developed an aneurysm of the thoracic arch of aorta requiring surgical intervention. The pedigree of family A is shown in figure 1A; and pedigrees for families B, C and G are provided as supplementary material. Eleven/15 (73.3%) patients had livedo racemosa. Neurologic manifestations were present in 8/15 patients (53.3%); central nervous system (CNS) involvement in 6 patients; and peripheral nervous system (PNS) involvement in 2 patients. Renovascular involvement was present in 3 patients (patients D, E and F), with renal arterial aneurysms and other features of medium vessel vasculitis demonstrated on catheter arteriography (Figure 1B and see below). Seven/15 (46.6%) subjects had immune deficiency: lymphopenia in 6/7 patients; and low immunoglobulin in 6/7 patients, 2 of whom required immunoglobulin replacement (Table 1).

Histopathological and angiographic findings

Six/10 of the symptomatic DADA2 patients had tissue biopsy performed (Supplemental Table S-2). This revealed evidence of vasculitis in 4/6. Eight patients had at least one mode of angiography performed (catheter digital subtraction arteriography, magnetic resonance angiogram, or computed tomography angiography). This was suggestive of vasculitis/vasculopathy in 7/8 patients (Supplemental Table S-2).

Treatment

Nine/10 patients required active treatment with anti-TNF-α, with or without daily prednisolone and/ or an alternative disease modifying anti-rheumatic drug (DMARD; Table 1). The indication for use of anti-TNF-α was insufficient therapeutic response to previous treatments (Table 1). Of note, patient B2 developed a fourth cranial nerve palsy 6 weeks after stopping colchicine, and approximately 8 months after obtaining a genetic diagnosis of DADA2. Treatment with adalimumab, high-dose corticosteroids, and 5 mg per kilogram of daily aspirin (after excluding intracerebral haemorrhage) resulted in complete resolution of his neurological symptoms and normalization of inflammatory markers within 7 days. Patient G-2 had a recurrent stroke 2 months after starting tocilizumab, prompting the clinician to convert this patient and his sibling (patient G-1) to adalimumab. At the time of writing, six months later, both patients remain in remission.

Identification of *CECR1* mutations

Mutations in *CECR1* identified in each subject are shown in Table 1. The mutations were initially identified by WES in 4 patients (A-5, B-1, B-2, and C-1). Sanger sequencing was performed in first-degree relatives of every patient (excluding the parents in family G); and in the extended family members of family A, where DNA was available. This revealed 5 subjects with predicted damaging homozygous or compound heterozygous *CECR1* mutations with minimal or no symptoms in family A and C, with age range 4-42 years (Table 1). In family A, the homozygous c.752C>T: p.Pro251Leu mutation was identified in the currently asymptomatic siblings, the mother, and maternal uncle. In family C, the compound heterozygous p.Pro251Leu and c.-114DelC (NM-001282227) combination was also identified in one currently asymptomatic sister, whose only clinical feature was mild livedo racemosa affecting the palms.

Sanger sequencing was performed in 9 patients with a clinical diagnosis of PAN, but with clinical suspicion of DADA2; mutations in *CECR1* were identified in 5/9 of these patients (D, E, F and G-1 & -2).

All identified mutations have been previously described in DADA2 patients (1,2) apart from the novel mutation c.144 G→Deletion in patient E causing a frameshift and insertion of a premature stop codon. The c.-114DelC (NM-001282227) in patients C-1 and C-2 has a prevalence of 4% in the population in the UK according to the SP6500 database (4). However, it affects the open reading

frame of the mRNA which is highly likely to interfere with protein translation, as confirmed by gene expression studies (below).

mRNA expression

CECR1 mRNA expression in all subjects with DADA2 who were tested with this assay (n=8, Table 1) was significantly lower than healthy controls (*p* value= 0.0016; figure 2A). *CECR1* mRNA expression of healthy adult controls was also significantly lower than healthy paediatric controls (*p* value = 0.0486). Of note, mRNA expression was confirmed to be low in subjects C-1 and C-2 who were compound heterozygous for the known Pro251Leu mutation, and the c.-114DelC variant.

Adenosine deaminase2 enzyme activity assay

ADA2 enzyme activity assay was measured in plasma from: symptomatic DADA2 patients (n=7); asymptomatic DADA2 subjects (n=4); adult carriers of *CECR1* mutations (n=5); sporadic cPAN patients without *CECR1* mutation (n=3; 3 males, median age 15 years, range 13-17 years); healthy paediatric controls (n=22; 13 males; median age 5 years, range 0.3 – 16 years); and healthy adult controls (n=20; 8 males, median age 30 years, range 20–44 years).

Results of the ADA2 enzyme activity assay are shown in Figure 2A. DADA2 subjects (irrespective of symptomatology) had lower ADA2 enzyme activity with median of 0.6483 unit/litre (range 0–3.35), compared to healthy paediatric

controls (median 10.63 unit/litre, range 1.73–31.45; $p<0.0001$), and cPAN patients without *CECR1* mutation (median 9.53 unit/litre, range 5.6-12.4; $p=0.0108$). Enzyme activity in adult carriers was not significantly different from healthy adult controls. Healthy paediatric controls had significantly higher enzyme activity than healthy adult controls ($p< 0.0001$; figure 2B).

DISCUSSION

We describe the phenotype and genotype of 15 subjects with DADA2, caused by loss of function mutations in *CECR1* which encodes for the ADA2 enzyme, resulting in an autoinflammatory and vasculopathic syndrome resembling PAN. Our series confirms the neurological phenotype of DADA2 described by others (Table 2); we also described a similar cutaneous phenotype with the exception of erythema nodosum, which we did not observe in our series. One patient had coarctation of the aorta; since congenital heart disease is not previously been described in DADA2, it remains to be established if this is truly part of the DADA2 phenotype or not. We emphasize a range of disease severity from limited cutaneous vasculitis in some subjects, to severe multi-systemic vasculitis in other individuals. One third (5/15) of our cases are currently asymptomatic, never having received treatment, at face value a novel finding in our series. The phenotypic manifestations of all the DADA2 patients in our series included cutaneous vasculitis (including livedo racemosa, ulceration, and ischaemia); neurologic involvement (53.3%) and lymphopenia and/or

hypogammaglobulinemia (7/15, 46.6%). Four/15 subjects fulfilled classification criteria for systemic cPAN (5); 5/15 (33.3%) had an initial clinical diagnosis of cutaneous PAN; one patient (subject C1) had an unclassified vasculitis; and 5/15 (33.3%) were asymptomatic, referred hereafter to as pre-symptomatic DADA2 (Table 1). Potentially of considerable diagnostic and prognostic importance, however, is that all 15 subjects had decreased enzymatic function of serum ADA2, and correspondingly low *CECR1* mRNA expression.

The 5 pre-symptomatic DADA2 subjects (age range 4-42 years old) were detected through screening of relatives of index cases. Four/5 had no evidence of systemic inflammation, lymphopenia, or hypogammaglobulinaemia (Table 1); data on one case (A-2) were not available regarding these laboratory parameters (Table 1). At face value, our description of these 5 asymptomatic cases is novel compared to other series; however, Elkan et al (1) have reported patients with DADA2 who presented with first symptoms in adulthood (18-59 years old). Therefore, with longer follow-up, some of these asymptomatic cases may develop symptoms of DADA2. As such, we recommend screening of all unaffected siblings of index cases by Sanger sequencing of *CECR1*, and serum ADA2 enzyme activity. From an ethical perspective we suggest that this screening approach is warranted since although it is unclear what factors trigger development of disease, the first presentation may be severe, disabling, or even life-threatening (such as stroke).

Pre-symptomatic molecular diagnosis of DADA2 allows early intervention with immunosuppression in the event of an acute presentation, and thus satisfies a general principle of genetic screening that knowledge of the genetic mutation would affect medical management. This indeed was the case for one of our series (subject B2) who developed a brain stem stroke and fourth cranial nerve palsy 6 weeks after stopping colchicine (Table 1); we rapidly commenced adalimumab plus high-dose corticosteroids and aspirin, which resulted in complete resolution of his neurological symptoms within 7 days. That said, it may be many decades before symptoms develop; pre-symptomatic genetic diagnosis of DADA2 during childhood therefore requires careful genetic counselling since not all families may elect to have this due to the significant burden of uncertainty regarding the prognosis. Since treating all pre-symptomatic patients with lifelong anti-TNF-α is ethically challenging, and economically questionable for third party payers, we currently recommend close and regular follow up of pre-symptomatic subjects. A biomarker to predict disease onset in pre-symptomatic cases is clearly urgently needed.

The serum ADA2 enzyme activity in all DADA2 subjects was significantly lower than healthy paediatric controls, and sporadic cPAN patients (n=3) without *CECR1* mutations. Interestingly, the enzyme activity of healthy paediatric controls was significantly higher than healthy adult controls, as was *CECR1* mRNA expression (Figure 2). This finding could suggest that ADA2 plays an important vaso-protective role in health, particularly-so in the young. One obvious

hypothesis to explain that is that ADA2 could act as a modulator of vascular inflammation triggered by intercurrent infection, which is much more common in the young. Of particular importance in this context was our observation of a lack of difference between DADA2 individuals and healthy adult controls; this has important practical implications in terms of the reference range used for the interpretation of ADA2 activity as a screening test for DADA2. Future work now needs to focus on how ADA2 operates at the physiological level in health and in ageing, and to properly examine the complex relationship between genotype, age, mRNA expression and ADA2 activity.

In our series, 9/10 symptomatic DADA2 patients required treatment with anti-TNFα; the main indication for this was inadequate therapeutic response to other DMARDs, and/or inability to wean corticosteroids. One important autoinflammatory feature of DADA2 is skewing towards M1 pro-inflammatory macrophages, causing dysregulated pro-inflammatory cytokine secretion including TNF-α and interleukin-1β (2). Thus, anti-TNF-α treatment appears to be effective, and is a logical choice for DADA2. Although studies in ANCA vasculitis favour the use of monoclonal antibodies over etanercept, published experience thus far does not obviously suggest that this is true for patients with DADA2, since at least 14 patients have received etanercept with seemingly favourable results (Table 2; 1,2,6,7). Less is known about IL-6 in DADA2, although tocilizumab could also be effective. However, subject G-2 developed a stroke 2 months after initiation of tocilizumab, and is currently stable on adalimumab. It is

unclear what role haematopoietic stem cell transplantation (8), gene therapy, or enzyme replacement (for example by regular plasma infusions) (9) might play in the treatment for DADA2 in the future.

In summary, we provide a description of the expanding phenotype and genotype of DADA2, and emphasise the fact that pre-symptomatic cases also demonstrate low levels of ADA2 enzyme activity, comparable to symptomatic DADA2 patients. It is currently unclear what triggers the full disease expression. We speculate that possible precipitating factors such as infection and/or other environmental influences could trigger disease expression. We have established tentative normative ranges for adult and paediatric ADA2 function, but we suggest there is a need for a much larger normative dataset for children and adults for this to be used with confidence as a screening test for DADA2. Whilst we provide age-related reference ranges for ADA2 enzyme activity, our data are too limited to provide didactic recommendations regarding screening strategies for this rare monogenic autoinflammatory disease. That said we recommend considering genetic screening and ADA2 enzyme activity in patients with familial vasculitis, recalcitrant PAN, and early-onset PAN-like disease (under age of 5 years).

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Table 1 Summary of clinical characteristics, treatment and genotype of 15 DADA2 patients

Patient	Sex/ Ethnicity	Initial Clinical Diagnosis	Age at genetic diagnosis (years)	Age at disease onset (years)	Highest CRP (mg/L) (normal <5)	Neurological involvement	Cutaneous involvement	Other features	Previous Treatments**	Current Pred dose (mg/kg)	Current Treatment (s)	CECR1 mutation (s)	mRNA expression tested
A-1	F/ Iraqi	Asymptomatic	42	NA	8	NA	NA	No systemic inflammation, No lymphopaenia, Normal Igs	NA	NA	Nil	Hom. c.752C>T: p.Pro251Leu	Y
A-2	M/ Iraqi	Asymptomatic	34	NA	Not yet tested	NA	NA	Not yet tested	NA	NA	Nil	Hom. c.752C>T: p.Pro251Leu	N
A-3	M/ Iraqi	Cutaneous PAN	19	10	12	ǂLower limb #UMN signs	Livedo racemosa	No	Colchicine	Nil	Nil	Hom. c.752C>T: p.Pro251Leu	Y
A-4	M/ Iraqi	Asymptomatic	17	NA	<1	NA	NA	No systemic inflammation, No lymphopaenia, Normal Igs	NA	NA	Nil	Hom. c.752C>T: p.Pro251Leu	N
A-5	M/ Iraqi	PAN	15	3	62	Axonal polyneuropathy	Livedo racemosa, Peripheral Ischaemia	Lymphopaenia low IgG	IV CYC , Pred 1 mg/kg/day	Nil	AZA, infliximab, Aspirin	Hom. c.752C>T: p.Pro251Leu	Y
A-6	F/ Iraqi	Asymptomatic	4	NA	<5	NA	NA	No systemic inflammation, No lymphopaenia, Normal Igs	NA	NA	Nil	Hom. c.752C>T: p.Pro251Leu	N

B-1	F/ Indian	Cutaneous PAN	8	5	17	No	Livedo racemosa, Nodular rash	▾Systemic inflammation	Colchicine	Nil	Adalimumab	Hom. c.139G>C: p.Gly47Arg	Y
B-2*	M/ Indian	Cutaneous PAN	6	5	24	*Brain stem stroke	Livedo racemosa, Nodular rash	▾Systemic inflammation	Colchicine	1	Adalimumab, aspirin	Hom. c.139G>C: p.Gly47Arg	Y
C-1	M/ Caucasian	Unclassified vasculitis	16	10	15	No	Peripheral ischaemia; livedo racemosa	Low IgM Coarctation of aorta	Nil	0.25	Adalimumab, aspirin	Compound het. c.752C>T: p.Pro251Leu and c.-114DelC	Y
C-2	F/ Caucasian	Asymptomatic	5	5	5	NA	Mild livedo racemosa of the palms	No systemic inflammation, No lymphopenia, Normal Igs	Nil	Nil	Nil	Compound het. c.752C>T: p.Pro251Leu and c.-114DelC	Y
D	M/ Caucasian	PAN	22	2	36	Intracerebral haemorrhage	Livedo racemosa	Low IgG, Lymphopenia Hypertension right optic nerve atrophy	Pred 2 mg/kg/day, IV CYC	0.25	AZA 1 mg/kg/day, IVIG; Infliximab, Aspirin	Compound het. c.2T>c and c.506 C>T: p. Arg169Gln	N
E	F/ Caucasian	PAN	11	6	67	Brain stem stroke	Livedo racemosa	Low IgG, Lymphopenia Hypertension	Pred 0.5 mg/kg/day, IV CYC followed by AZA 2 mg/kg/day, MMF, Rituximab	Nil	AZA 2 mg/kg/day, Infliximab, Aspirin	Compound het. c.2T>C and c.144delG: p.Arg49GlyfsX4	N
F	F/ Caucasian	PAN	24	6	26	Intracerebral haemorrhage	Peripheral ischaemia; livedo racemosa	Lymphopenia, Hypertension, Reno-vascular arterial aneurysms	Pred 0.5mg/kg/day, IV CYC followed by AZA 2 mg/kg/day	Nil	MMF 25 mg/kg/day /Infliximab	Compound het. c.506C>T: p.Arg169Gln and c.1358A>G: p.Tyr453Cys	N

G-1	M/ Caucasian	PAN	11	3	32	Basal ganglia stroke	Livedo racemosa	Lymphopenia, Low IgM, ♥Systemic inflammation	Pred 2 mg/kg/day, AZA, MTX Tocilizumab	Nil	Adalimumab	Hom. c.506C>T: p.Arg169Gln	N
G-2	M/ Caucasian	PAN	12	5	15	Intracerebral haemorrhage	Livedo racemosa	Lymphopenia, Low IgM, ♥Systemic inflammation	Pred 2 mg/kg/day, IV CYC Tocilizumab	Nil	Adalimumab	Hom. c.506C>T: p.Arg169Gln	N

Patients from the same family are grouped by letter**.** *Patient B2 developed 4th cranial nerve palsy 6 weeks after stopping colchicine, and approximately 8 months after obtaining a genetic diagnosis of DADA2. Treatment with adalimumab, high-dose corticosteroids, and 5 mg per kilogram of daily aspirin resulted in complete resolution of his neurological symptoms and normalization of inflammatory markers within 7 days. Patient G-2 had a recurrent stroke on tocilizumab, prompting the clinician to convert this patient and their sibling (patient G-1) to adalimumab. **All of these treatments failed to control the disease adequately. ♥Systemic inflammation: persistently elevated ESR, CRP, and Serum amyloid A. #UMN: upper motor neurone (┼brisk ankle reflexes, excessive ankle clonus, but normal brain and spinal cord imaging); AZA: azathioprine, CRP: C reactive protein, CYC: cyclophosphamide, DADA2: deficiency of adenosine deaminase 2, ESR: erythrocyte sedimentation rate, F: female, Het: heterozygous, Hom: homozygous, IV: intravenous, M: male, Mg/kg/day: milligram/kilogram/day, MMF: mycophenolate mofetil, NA: not applicable, PAN: polyarteritis nodosa, Pred: prednisolone.

	Our series (N=15)	Zhou et al[2] (N=9)	Elkan et al[1] (N=24)	Batu et al[7] (N=6)
Clinical manifestations	**N (%)**			
Neurological involvement (total)	8 (53.3)	9 (100)	15 (62.5)	3 (50.0)
Central nervous system	6 (40.0)	9 (100)	5 (20.8)	3 (50.0)
- Intracerebral haemorrhage	3 (20.0)	3 (33.3)	1 (4.2)	NR
- Stroke	3 (20.0)	8 (88.9)	5 (20.8)	3 (50.0)
Peripheral nervous system	2 (13.3)	NR	10 (41.7)	NR
Cutaneous involvement (total)	11 (73.3)	8 (88.9)	16 (66.7)	6 (100.0)
Livedo racemosa	11 (73.3)	8 (88.9)	16 (66.7)	5 (83.3)
Peripheral Ischaemia	3 (20.0)	NR	5 (20.8)	2 (33.3)
Erythema nodosum	0 (0)	2 (22.2)	1 (4.2)	4 (66.7)
Immunological involvement	7 (46.6)	5 (55.6)	NR	2 (33.3)
Lymphopaenia	6 (40.0)	0 (0)	NR	NR
Hypoglobulinaemia	6 (40.0)	5 (55.6)	NR	2 (33.3)
Gastrointestinal involvement	4 (26.7)	8 (88.9)	7 (29.2)	6
Renal involvement	1 (6.6)	2 (22.2)	7 (29.2)	1 (16.7)
Hypertension	3 (20.0)	1 (11.1)	7 (29.2)	NR
Ophthalmologic involvement	1 (6.6)	5 (55.6)	NR	3 (50.0)
Asymptomatic	5 (33.3)	0 (0)	0 (0)	0 (0)
Congenital heart disease	1 (6.6)	NR	NR	NR
Treatment with Anti-TNF	**9**	**6**	**10**	**3**
Etanercept	0	6	5	3
Infliximab	4	0	2	0
Adalimumab	5	0	3	0

Table 2: Clinical features and anti-TNF treatment received by our series and 3 other published series (containing >3 cases). NR: not reported.

Figure legends

Figure 1 A**:** Family pedigree and summary of sequenced genotype of p.P251 on CECR1 gene in the extended family A. All genotypes identified by Sanger sequencing. WT, wild type; P251L/WT is heterozygous variant and P251L/P251L is homozygous mutation. B: digital subtraction angiogram of left kidney of patient F shows multiple aneurysmal dilatations, and other caliber variation of the medium-small intra-renal arteries, C: computer tomography of brain of patient F revealing intracerebral haemorrhage. Formal catheter cerebral arteriography (not shown) did not reveal any abnormality, consistent with lacunar (small vessel) haemorrhagic stroke. D: Livedo racemosa in subject C1 and E. lower limb ulcer in subject C1.

Figure 2 A: CECR1 mRNA expression was reduced in 7 children and 1 adult (asymptomatic) with DADA2 compared with 2 healthy paediatric ($p=0.016$) and 4 healthy adult controls B: The adenosine deaminase 2 enzyme activity was reduced in 11 patients with DADA2 compared to 22 healthy paediatric controls ($p<0.0001$) and 3 children with sporadic PAN (CECR1 wild-type, $p=0.01$); there was no significant difference between adult carriers and healthy adult controls ($p=0.2390$). Healthy paediatric controls had significantly higher enzyme activity than healthy adult controls ($p< 0.0001$)