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Deficiency of adenosine deaminase 2 (DADA2) in Adults and Children: Experience from India

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Author Contribution

AS, GN, SanJ and PYL conceived and designed the study. AS, GN, VikS, SakJ, AD, VD, PB, VisS, SB, CK, VG, PPC, SM, RD, BS, SK, RB, PG, KS, MS, MR, RN, RWM, VC, AA, RH, AG, MG, ZH, JW, RJ, VN, RK, SanJ, JIA, EPC, MSH, IA, QZ and PYL contributed to acquisition of data. AS, GN, DPM, VA, EPC, QZ, PYL contributed to analysis and interpretation of data. AS, GN and PYL drafted the manuscript and all authors edited and revised the manuscript critically. All authors approved the final version of the manuscript for submission.

Competing Interests

The authors declare no conflict of interest related to this work.

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Abstract

Objective: Deficiency of adenosine deaminase 2 (DADA2) is a potentially fatal monogenic syndrome characterized by variable manifestations of systemic vasculitis, bone marrow failure and immunodeficiency. Most cases are diagnosed by paediatric care providers given the typical early age of disease onset. We aim to describe the clinical phenotypes and treatment response of adults as well as paediatric DADA2 patients in India.

Methods: We conducted a retrospective analysis of DADA2 patients diagnosed at various rheumatology centres across India. The clinical characteristics, diagnostic findings and treatment response of all the subjects were analysed.

Results: We confirmed 33 cases of DADA2 between April 2017 and March 2020. Unlike previous studies, nearly half of the cases presented during adulthood. All symptomatic patients exhibited features of vasculitis while constitutional symptoms and anaemia were more common in paediatric patients. Cutaneous and neurologic involvement were common and 18 subjects had at least one stroke. In addition, we expand the clinical spectrum of DADA2 by describing novel features including pancreatic infarction, focal myocarditis and diffuse alveolar haemorrhage. Tumour necrosis factor inhibitors (TNFi) were initiated for 25 patients. All measured disease manifestations showed marked improvement after initiation of TNFi and disease remission was achieved in 19 patients. Two cases were complicated by tuberculosis infection and two deaths were reported.

Conclusions: We present the first case series of DADA2 patients from India. We highlight the presentation of DADA2 in adults and raise awareness of this syndrome for both adult and paediatric care providers.

Keywords

Adenosine deaminase 2; DADA2; Vasculitis; TNF inhibitors

INTRODUCTION

Adenosine deaminase 2 (ADA2) is an extracellular enzyme secreted by activated monocytes, macrophages and dendritic cells. Although its physiologic function is not entirely clear, ADA2 promotes the differentiation of monocytes to macrophages and may also acts a growth factor for endothelial cells and haematopoietic cells.[1–3] ADA2 is encoded by *ADA2* gene (previously known as *CECR1,* Cat Eye syndrome Chromosome Region 1 gene) on chromosome 22q11.1.[4]

Deficiency of ADA2 (DADA2) results from biallelic mutations in *ADA2*.[5, 6] Since the first description in 2014, case reports and series from different countries have expanded the clinical spectrum of the disease.[7–11] Common clinical features of the disease include recurrent fever, skin ulcers, livedo reticularis, early-onset stroke, peripheral neuropathy, hypogammaglobulinemia, cytopenias and elevated acute phase reactants.[5–11] These features led to the recognition of DADA2 as a monogenic form of polyarteritis nodosa (PAN) that presents in early childhood. Although a few cases of DADA2 in adults have been

described,[6, 12] little is known regarding the clinical manifestations and treatment outcomes in patients with adult-onset DADA2.

Anti-tumour necrosis factor (TNF) therapy is established as the treatment of choice for DADA2 with remarkable efficacy in preventing stroke.[13] However, how other clinical manifestations respond to this treatment has not been described in detail and there are cases that do not respond to TNF inhibitors (TNFi).[14] Here, we describe the clinical features, genotypes and treatment outcomes of 33 subjects with DADA2 in India. Unlike previous cases series, nearly half of the patients in our cohort were adults at the time of disease onset. We compare the clinical manifestations in patients presenting during childhood vs. adulthood, and report the response of various clinical manifestations to TNFi.

MATERIALS AND METHODS

Subjects and study design

We conducted a retrospective analysis of patients diagnosed with DADA2 at different rheumatology centres across India. To recruit the patients, a request was sent to all the members of Indian rheumatology association to contribute data of DADA2 patients diagnosed at respective centres. Diagnosis of DADA2 was established by ADA2 enzyme activity assay and/or *ADA2* gene sequencing depending upon the logistic availability at each centre. The clinical details including presenting manifestations, organ involvement, laboratory investigations, treatment approaches and response to therapy in all subjects were noted in predetermined proforma. Results of tissue biopsy and radiographic imaging including computed tomography (CT), magnetic resonance imaging (MRI) and angiography of involved organs were also collected. Two patients were included in previous reports.[14, 15] Demographics and collection of clinical data are described in detail in Supplemental methods. Clinical remission was defined by 1) absence of active systemic inflammation, 2) absence of active organ-specific vasculitis, and 3) improvement or stabilization of prior organ damage related to DADA2. Treatment outcomes in various individual manifestations were recorded as resolved (complete improvement), improved (partial improvement), persisting activity (ongoing activity) and relapse (recurrence after initial resolution) or new onset features. This study was approved by the Institute ethics committee of PGIMER, Chandigarh, India. A waiver of patient consent was granted by the Institute Ethics Committee for the retrospective analysis.

ADA2 activity assay

ADA2 activity measurement from plasma or dried plasma spots was performed using spectrophotometric assay as was described previously.[5, 16] This assay quantifies the adenosine-dependent generation of ammonia in the presence of a selective inhibitor of ADA1, EHNA (erythro-9-Amino-β-hexyl-α-methyl-9H-purine-9-ethanol hydrochloride). EHNA was purchased from Cayman Chemicals and all other reagents were purchased from Sigma Aldrich. The kinetics of each reaction were analyzed using a Biotek Synergy Hybrid Microplate Reader.

***ADA2* gene mutation analysis—**Ten exons of ADA2 gene was amplified using KOD FX high success-rate DNA polymerase (#KFX-101) and PCR product of each amplicon was purified using Axygen AxyPrep DNA Gel Extraction Kit (#AP-GX-250) following the manufacture's instruction. Sanger sequencing was conducted as previously described.[5, 17] Sanger sequencing results were analysed using Sequencher 5.4.6. Structural modelling was performed using PyMOL and UCSF Chimera software.[18] Construction of pcDNA3.1 plasmid encoding wild-type or mutant ADA2 with C-terminal Myc tag was performed as previously described.[17] The list of mutations and primer pairs used to generate mutant constructs are available in supplemental table S1. Plasmids were transfected into 293T cells using Fugene 6 (Promega). ADA2 expression in cell lysate and ADA2 activity in the culture medium were quantified after 3 days. Each mutant was analysed by at least three independent experiments and measurements were normalized to the activity of wildtype ADA2.

Western blotting

ADA2 was detected using a monoclonal antibody to Myc-tag as described.[17] Transfected cells (5×10^5) were resuspended in 100 μl of Laemmeli's buffer with 2-mercaptoethanol. SDS-PAGE was performed using an 8% polyacrylamide gel. After transfer to PVDF membrane and blocking with 5% dry milk, primary antibody to Myc tag (Clone 9E10; Biolegend) or GAPDH (Clone D4C6R; Cell Signaling Technology) was applied for 1 hour. Membranes were washed in TBS with 0.1% Tween 20 and HRP-conjugated goat anti-mouse IgG (1:5000; Cell Signaling Technology) was used for detection. Images were acquired using a Bio-Rad ChemiDoc system.

Statistical analysis

Median with interquartile range was used to express continuous variables while percentages and proportions were used to express categorical variables. The differences between two groups were analyzed using the Mann-Whitney U test and Chi-square was used for comparison of categorical variables. Multiple linear regression analysis was performed to predict the factors associated with delay in diagnosis. All tests were two-sided, and $p < 0.05$ was considered significant. Statistical analyses were performed using Prism 8.0 software (GraphPad Software, La Jolla, CA).

RESULTS

Demographic characteristics

Between April 2017 and March 2020, a total of 124 patients were tested for DADA2 by enzymatic activity assay and/or *ADA2* gene mutation analysis. Out of these 124 patients we established the diagnosis of DADA2 in 33 patients from 28 unrelated families. Eighteen patients were from North India, nine were from South India, five were from West India and one patient was from Syria. Thirteen of the 18 northern India patients and three of the five western India patients belonged to Agarwal/Jain community. All patients presented with features of systemic vasculitis, except for one infant who initially presented with pure red cell aplasia (PRCA) and two individuals who were diagnosed by means of screening of first degree relatives of previous confirmed cases. Although these two subjects were

asymptomatic at the time of diagnosis, both had past history suggestive of DADA2 manifestations including skin vasculitis (n = 2) and central retinal artery occlusion (n = 1).

Biallelic mutations in *ADA2* gene were confirmed by sequencing in 22 patients that submitted a DNA sample. The remaining patients were diagnosed based on near-absent plasma ADA2 activity (Figure 1A). Demographics and clinical manifestations of the cohort are summarized in Table 1. Distinct from the typical childhood-onset pattern of DADA2 established by previous studies (median age: 4 years), 16 / 33 (49%) subjects in this cohort had disease onset during adulthood (> age 16 years), and the overall median age of onset was 15 years (Figure 1B). Median time to diagnosis from initial presentation was 52 months. A multiple linear regression analysis was performed to predict the time to diagnosis from gender, age at disease onset, constitutional symptoms, CNS, PNS, GI, renal, skin and joint involvement and presence of stroke and mesenteric ischemia. None of the variables predicted the time to delay in diagnosis among these patients (Supplemental Table S2).

Clinical features of DADA2 patients

Vasculitis that mimics PAN was the predominant phenotype in our cohort. The nervous system and skin were most commonly involved organs, affecting 79% and 73% of patients, respectively (Table 1). More than half of patients had at least one ischemic or haemorrhagic stroke. Constitutional symptoms were noted in 55% of patients. Other common organ-specific manifestations included those affecting the renal system (49%), gastrointestinal tract (46%) and joints (30%). Testicular pain was present in 17% of males and pregnancy loss was noted in 2 out of 10 females. Figures 1 (C–F) illustrate the protean clinical manifestations of DADA2 including small bowel obstruction, end organ infarction and visceral vessel aneurysms.

Rare manifestations in our cohort that have not been previously described in DADA2 include pancreatic infarct (Figure 1D), diffuse alveolar haemorrhage with respiratory failure (Figure 1G) and focal myocarditis with bradycardia (not shown; each found in one patient). Supplemental Table S3 shows the major clinical manifestations of individual patients in our cohort.

Laboratory studies revealed anaemia in 27% of patients and leukopenia was noted in one subject. Hypogammaglobulinemia was not found in this cohort. Most patients had elevated ESR (median 49 mm/hour, range 10–155 mm/hr) and CRP (median 42 mg/L, range 0.39–140 mg/L).

The frequency of most DADA2 clinical features were similar between childhood-onset and adult-onset cases (Table 1). Significant differences from univariate analysis showed that adult-onset DADA2 patients had less constitutional symptoms (38% vs. 71%, p = 0.037) and hematologic manifestations (12% vs. 47%, p = 0.031). Supplemental Table S4 compares the features of our patients with published cohorts for DADA2 and PAN. Overall, the clinical features of our cohort resembled the described manifestations of DADA2 with only mild differences between adult and paediatric cases. CNS involvement appears to be a major distinguishing feature between DADA2 and PAN in adults. Whereas 56% of our adult-onset

DADA2 cases experienced at least one stroke, <5% of 384 patients with adult-onset PAN exhibited stroke or other CNS involvement.[19]

Radiographic and histopathology results

Computed tomography(CT) or magnetic resonance imaging(MRI) of the brain was performed in 22 patients and abnormal findings were noted in all but two. Nine patients had only ischemic infarcts, two had only intracranial haemorrhage and five exhibited both findings (concurrently in two cases and as separate events in three patients). Three cases displayed aneurysms of cerebral vessels (Figure 2A) and one showed meningeal enhancement and multiple hyperintensities suggestive of posterior reversible encephalitis syndrome (PRES; Figure 2B). All patients except one underwent abdominal ultrasonography (n=28) and/or abdominal CT (n=23). Eight patients had renal infarcts (Figure 1C) and one patient had renal, splenic and pancreatic (Figure 1D) infarcts. Perinephric haematoma was noted in two patients. Hepatomegaly, splenomegaly and fatty infiltration of liver were noted in three patients each. Mesenteric lymphadenopathy was noted in one patient. Features of mesenteric ischemia were noted in four patients and one patient had ascites. None of the patients had hepatic infarcts, focal nodular hyperplasia of the liver or features of portal hypertension. Vascular aneurysms affecting various branches of the abdominal aorta were found in nine patients (Figure 1E and 1F). Peripheral arterial narrowing or occlusion was noted in three patients (Figure 2C).

A total of 23 tissue biopsies were obtained from 20 patients. Overall, vasculitis of small or medium vessels was noted in 16 (70%) and vasculopathy in two (9%) biopsies. Among the 12 skin biopsies, vasculitis was noted in nine, while livedoid vasculopathy, septal panniculitis, and acne vulgaris were noted in one each. Out of 5 intestinal biopsies, two had vasculitis while one each had active ileitis and ischemic enteritis. Gastrointestinal ulcerations were noted in two patients (Figure 2D). Four nerve biopsies were obtained and all revealed evidence of vasculitis. Temporal artery biopsy in one patient showed active vasculitis while testicular biopsy from one male was inconclusive. Figure 2 (E–F) shows the presence of vasculitis and acute inflammation in the colonic biopsy of a patient with mesenteric ischemia. Taken together, these findings suggest DADA2 is a mimic of PAN in adults as well as children.

ADA2 activity and *ADA2* gene mutations

Plasma ADA2 activity was assessed in 20 patients and all exhibited near-absent levels that were significantly lower than the levels found in carriers with one mutation (Figure 3A). Among the 22 patients with genetic analyses, 16 patients were homozygous and six were compound heterozygous for pathogenic mutations in *ADA2.* Ten different ADA2 mutations were identified (Figure 3B), including 8 missense mutations and 2 splice-site mutations (supplemental Table S5). Most of these variants have been described previously.[5–7, 15, 20–22] The p.G47R variant was the most common allele in this cohort, found in 18 of 22 patients. In a sub-group analysis of patients with genetic testing, there was no difference in the clinical features or outcomes between patients with homozygous p.G47R alleles from patients with other mutations(data not shown). There was no difference in the clinical manifestations and treatment outcomes in patients with confirmed *ADA2* mutations versus

those without genetic studies. There was also no statistical difference in the rate of genetic testing between adults and children. One patient possessed two novel variants, p.H112Y and p.G321A, that affect the same amino acid residues as previously identified pathogenic variants (p.H112Q and p.G321E, respectively).[5, 23] The p.H112Y variant was predicted to disrupt zinc ion coordination within the ADA2 structure while p.G321A induced clashes with adjacent amino acids by structural modelling (Figure 3C). Confirming the pathogenicity of the *ADA2* mutations in this cohort, *in vitro* expression of the missense variants in 293T cells showed detectable protein expression but reduced ADA2 enzymatic activity in the supernatant (Figure 3D–E).

ADA2 mutations with residual enzymatic function (>3%) are associated with the vasculitis phenotype while null mutations are found in patients with severe hematologic manifestations of DADA2. [14] A similar correlation was noticed in our cohort as 19 / 22 patients with genetic diagnosis possessed at least one hypomorphic allele (G47R or L188V) with residual activity >3% (Supplemental Table S3 and S5). Insertions / deletions with frameshift and nonsense mutations associated with hematologic compromise were not found in our patients.

Treatment and outcomes

Before the diagnosis of DADA2, most patients were treated with high dose glucocorticoids (76% orally and 46% intravenously; Figure 4A). Seventy percent of patients received one or more immunosuppressant agents with cyclophosphamide(42%), azathioprine(24%), mycophenolate(21%) and methotrexate(18%) as the more common choices. Five patients received aspirin and four were given rituximab. The overall efficacy of theses interventions was suboptimal as most patients were glucocorticoid-dependent and many continued to have either persistent disease or relapse.

TNFi are widely considered the treatment of choice for the vasculitis / inflammatory phenotype of DADA2.[6,9,13] Once the diagnosis of DADA2 was established, 25 patients in our cohort (76%) were started on TNFi after ruling out tuberculosis infection. The fraction of patients that required glucocorticoids was reduced by about a half and most patients also discontinued the use of other immunosuppressive medications (Figure 4A). The initial choice of TNFi included adalimumab (n = 14), etanercept (n = 10) and infliximab (n = 1). One patient sequentially received all three agents as well as golimumab. Excluding this patient, biosimilar TNFi were used in 18 patients (75%) while innovator products were given to six patients (25%).

In the TNFi treated group, one patient expired during the first admission when the diagnosis was established. She had severe intestinal vasculitis at baseline that was resistant to high-dose glucocorticoids and cyclophosphamide. Her gastrointestinal symptoms improved transiently after starting adalimumab but the patient succumbed to sepsis three weeks later. Three patients discontinued TNFi. One patient discontinued treatment after six months as she developed active pulmonary tuberculosis without evidence of active features of DADA2 at her last follow up. Two patients discontinued TNFi after three months due to financial constraints. One of these patients initially responded well to adalimumab, but died due to gastrointestinal bleeding four months after discontinuing treatment; while the second patient had coronary artery disease requiring coronary angioplasty and stenting. All eight patients in

the non-TNFi group were offered TNFi but refused. Two had no features of active disease; two had relapsing disease but could not afford treatment; three were in remission with other treatment regimens and one patient was lost to follow up.

Among the remaining 21 patients maintained on TNFi (11 childhood-onset, 10 adult-onset), 19 achieved clinical remission. The response of various organ manifestations to TNFi is shown in Figure 4B. All ten categories of DADA2 features captured in our study either resolved or improved in most patients after treatment, without notable differences between 1) adults vs. paediatric patients, 2) the choices of TNFi, or 3) innovator products vs. biosimilars. Notably, these 21 patients had a combined 22 ischemic or haemorrhagic stroke events prior to anti-TNF therapy and one event to date since treatment initiation.

Persistent or relapsing disease and new manifestations were noted in two patients. One patient had a relapse of cutaneous vasculitis and new-onset foot drop while receiving biweekly adalimumab. These findings improved after adalimumab dosing was escalated to once weekly. The second patient suffered recurrent strokes, relapsing cutaneous vasculitis and neuropathy despite trials of multiple TNFi including etanercept, adalimumab, golimumab and infliximab. She had multiple relapses while maintained on these medications and her clinical course was further complicated by brief treatment cessation in the setting of active tuberculosis. Taken together, these data demonstrate the overall efficacy of TNFi for manifestations of vasculitis in both adults and children with DADA2. Cases of relapsing disease and treatment discontinuation also highlight the challenge of treating this complex syndrome.

DISCUSSION

In this study, we describe the clinical characteristics, genetic investigations and treatment response of 33 subjects with confirmed DADA2. This is the first case series on DADA2 from India. Our study highlights that onset of DADA2 is not restricted to young children and adult providers also need to recognize this monogenic syndrome. Given the wide age range of disease onset, we suggest that DADA2 should also be considered for adult PAN. Early diagnosis by sequencing or ADA2 activity assay followed by initiation of anti-TNF treatment can significantly improve the morbidity of this potentially fatal disease.

DADA2 was first recognized as a monogenic syndrome of early-onset stroke and systemic vasculitis that mimics PAN.[5, 6] Subsequent studies have noted that about ¼ of childhood PAN cases are due to DADA2.[9] The most striking difference between our patients and published cohorts is the age of disease onset (supplemental Table S4). In a recent review of 161 patients with DADA2, 77% of patients had disease onset before the age of 10 years.[24] Only a few adult cases have been described in the past and some had childhood disease onset with delayed diagnosis.[6, 12, 25] In contrast, more than 2/3 of subjects in our cohort exhibited disease onset after 10 years of age and about 1/3 presented after 20 years of age. The oldest subject in our cohort presented at 37 years of age with haemorrhagic stroke and skin ulcers. What triggers disease onset in DADA2 is not clear and some individuals with biallelic pathogenic variants can remain asymptomatic well into adulthood.[6, 12] Our cohort had a slight male preponderance with 69% males while the previous reports showed

the expected equal sex distribution for an autosomal recessive disease.[24] This discrepancy might reflect the health care seeking behaviour in our country.

Most of our patients exhibited features of PAN and the frequency of various manifestations is within the range described by previous series.[5–11] The clinical features are mostly comparable between childhood-onset and adult-onset cases, except for mild differences in the prevalence of constitutional symptoms and anaemia. The findings of diffuse alveolar haemorrhage, pancreatic infarct, focal myocarditis and PRES-like encephalopathy further expand the broad clinical spectrum of DADA2. A hallmark that distinguishes DADA2 from PAN is the prevalence of CNS disease. More than 50% of our patients had at least one episode of ischemic stroke or brain haemorrhage, compared to <5% CNS involvement in classic PAN.[19] Cytopenias and hypogammaglobulinemia are suggested as additional features that favour DADA2,[5, 26] although these features were generally absent in our patients, possibly due to referral bias, environmental differences and/or additional genetic modifiers.

Severe hematologic manifestations associated with DADA2 such as neutropenia, pancytopenia, immune mediated thrombocytopenia and lympho-proliferative disorders were not seen in our patients. This is consistent with the genotype-phenotype correlation recently described in DADA2: missense mutations with residual enzymatic activity (>3%) align with the vasculitis phenotype while variants with minimal residual function, including nonsense mutations and insertion / deletion mutations, are associated with severe hematologic compromise.[14] In line with this view, one child in our cohort developed early-onset PRCA and was found to have homozygous G358R mutations, a variant that displays minimal residual enzymatic activity and was previously described in patients with PRCA and bone marrow failure syndrome. [14, 21]

Our understanding of the pathophysiology of DADA2 remains incomplete as the function of ADA2 awaits further clarification. To date, more than 80 pathogenic variants have been linked to DADA2.[28] The commonest pathogenic variant in our cohort was p.G47R. The p.G47R variant is uncommon in the general population (minor allelic frequency: 1×10^{-4}in gnomAD database) but was found in ~1/10 individuals in the Georgian-Jewish community in Israel and Turkey.[6, 26, 29] Another interesting point noted in our cohort was the clustering of patients to the Agarwal/Jain community, with most of these patients harbouring the p.G47R variant. This is an endogamous community and genetic diseases like limb-girdle muscular dystrophy type 2A, megalencephalic leukodystrophy with cysts and hereditary fructose intolerance have been reported in this community.[30–33]

Even in the absence of randomized controlled trials, TNFi have emerged as the drug of choice for the treatment of DADA2.[6, 9] A recent study by Ombrello and colleagues revealed a drastic reduction in stroke risk after initiation of TNFi.[13] Based on this experience, we recommended initiation of TNFi in symptomatic patients immediately after the diagnosis of DADA2. We quantified the effects of TNFi on various disease manifestations and found remarkable improvement in all the measured parameters. The use of glucocorticoids was reduced and treatment with other immunosuppressants including cyclophosphamide and rituximab was no longer necessary.

Our experience showed that biosimilar TNFi are effective for DADA2 although no direct comparison with innovator molecules is available. While clinical improvement was seen in most cases, dose escalation of adalimumab was required for disease control in one case and another patient had relapsing disease despite trialling multiple TNFi. On the other hand, our data also illustrate the challenges of chronic TNFi therapy. Two patients developed active tuberculosis during treatment and one died from overwhelming sepsis. In addition, several patients were either not able to start or discontinued treatment due to financial limitations. One patient relapsed a few months after treatment cessation and succumbed to severe gastrointestinal haemorrhage.

The main limitation of our study is the retrospective nature of the study. Another limitation is the variability in diagnostic methods without complete information on *ADA2* mutations and ADA2 enzyme activity for all patients in our cohort. This was due to logistic limitations at different centres involved in the study. In addition, referral bias could have contributed to the paucity of hematologic and immunologic defects in our cohort as all patients were recruited from rheumatology centers.

In conclusion, this case series describes the clinical features, genetic findings and treatment outcomes of DADA2 patients in India. We show that disease onset during adulthood is common and clinical presentation may occur as late as the fourth decade of life. Rapid diagnosis and treatment with TNFi are important for successful outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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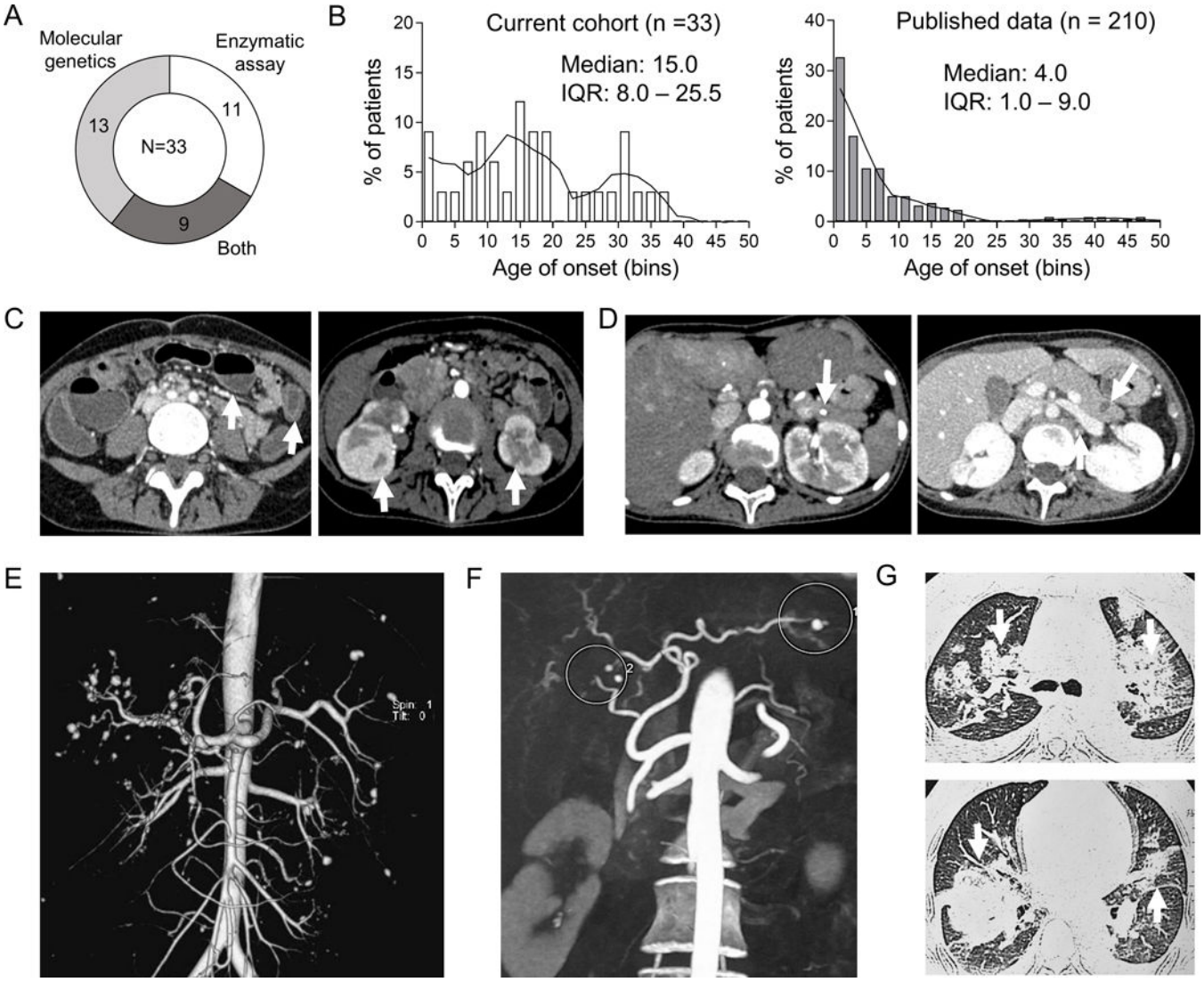

Figure 1. Clinical characteristics and disease manifestations of enrolled patients.

A) Distribution of methods utilized to confirm the diagnosis of DADA2. B) Comparison of disease-onset age in the current cohort (n = 33) with published cases (n = 210). Median and interquartile range (IQR) are indicated. C-G) Clinical manifestations of DADA2. C) Small bowel obstruction and bilateral renal infarcts in a 43-year-old male (CT scan). D) Micro-aneurysm (left) and an infarct (right) within the pancreatic body in a 17-year-old female (CT scan). E) Aneurysms of the left lower intercostal artery, hepatic, splenic, renal and mesenteric arteries in a 16 year-old-male (CT angiography, 3D reconstruction). F) Aneurysms of right hepatic artery (left circle) and splenic artery (right circle) in a 17-year-old female (CT angiography). G) Bilateral consolidation and surrounding ground glass opacities in a 38-year-old man with diffuse alveolar haemorrhage (CT scan).

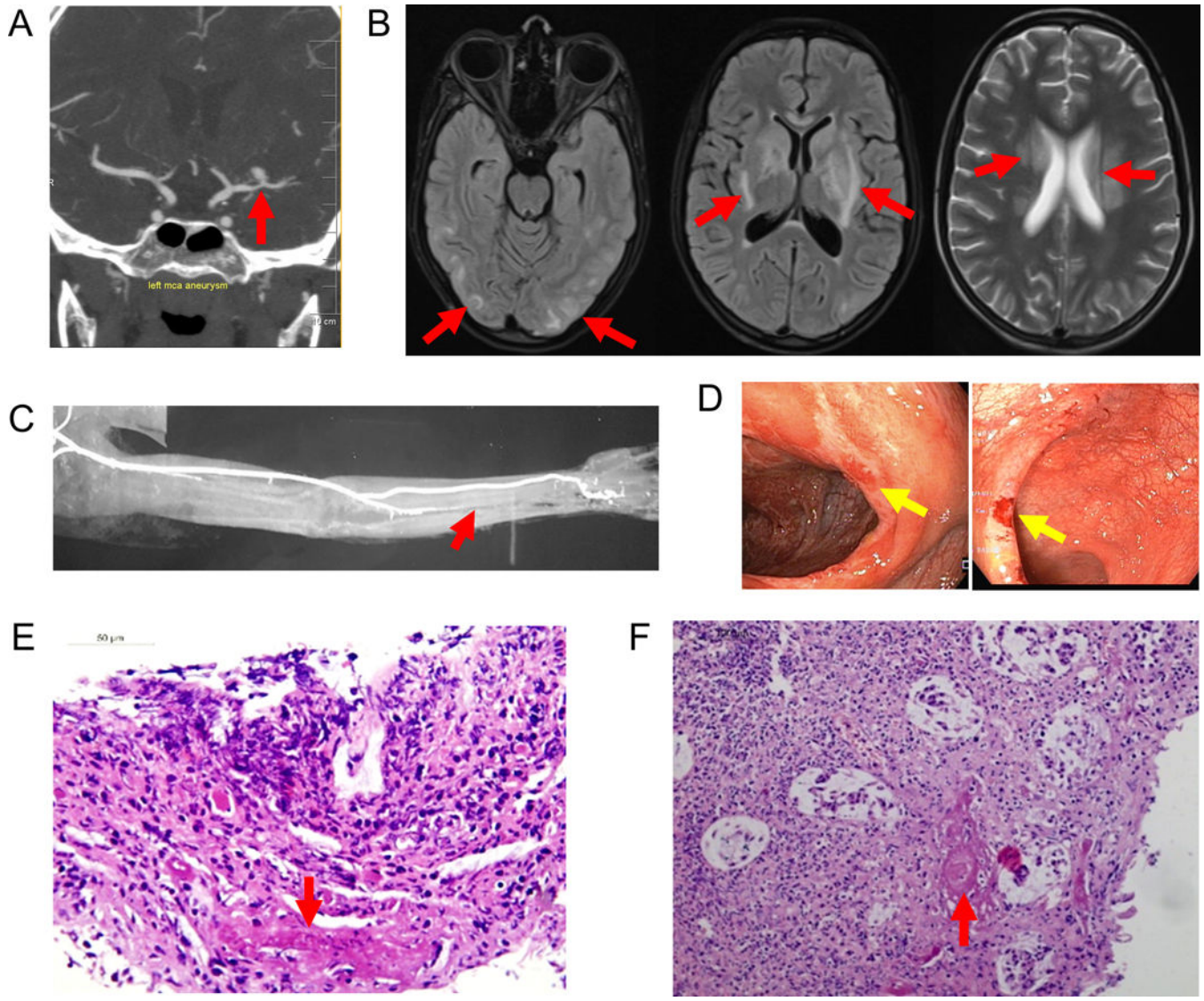

Figure 2. Radiographic and biopsy findings of end organ damage in DADA2.

A) CT angiography of intracerebral vessels in a 14-year-old male show aneurysm in the left middle cerebral artery. B) MRI images of the brain show areas of enhancement in the bilateral parietal subcortical white matter (left), bilateral basal ganglia, internal and external capsules (middle) and centrum semiovale (right) in a 17-year-old female. C) CT angiography of the left upper limb shows marked attenuation of the distal half of the left ulnar artery in a 35-year-old female. D) Endoscopy image of ulcers in the caecum (left) and rectum (right) in a 17-year-old female with gastrointestinal involvement. E-F) Haematoxylin and Eosin biopsy images (40X magnification) from a 17-year-old female with mesenteric ischemia. E) Acute ulcer with fibrinoid necrosis of arteriolar wall with neutrophil debris indicating arteritis. F) Mucosal injury and moderate chronic inflammation in the lamina propria along with acute vasculitis with thrombosis.

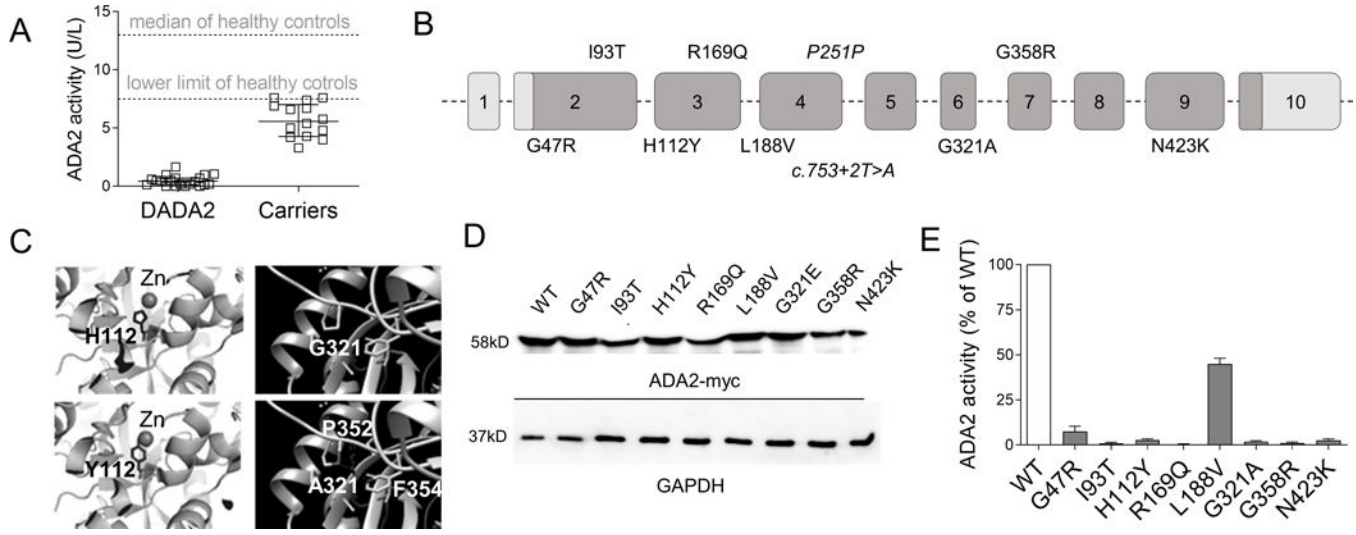

Figure 3. Confirmation of DADA2 by enzyme activity and genetic studies.

A) Comparison of plasma ADA2 activity in patients (n = 20) and carriers with monoallelic mutation (n = 8) using an established spectrophotometric assay. Median and lower limit of normal determined from > 200 healthy individuals are indicated. B) Listing of mutations according to their exon location. C) Structural modelling illustrates disruption of zinc ion coordination by p.H112Y variant (left) and clashes with nearby amino acids (within 5 angstroms) induced by p.G321A variant. D) Western blot of cell lysates from 293T cells transfected with wildtype and mutant ADA2 constructs. Top panel: ADA2 (detected by antibodies to Myc tag); lower panel: GAPDH loading control. E) Spectrophotometric quantification of ADA2 activity in the medium of transfected 293T cells. Bars represent the mean of three independent experiments. ADA2: Adenosine Deaminase 2; DADA2: Deficiency of Adenosine Deaminase 2; WT: Wild type.

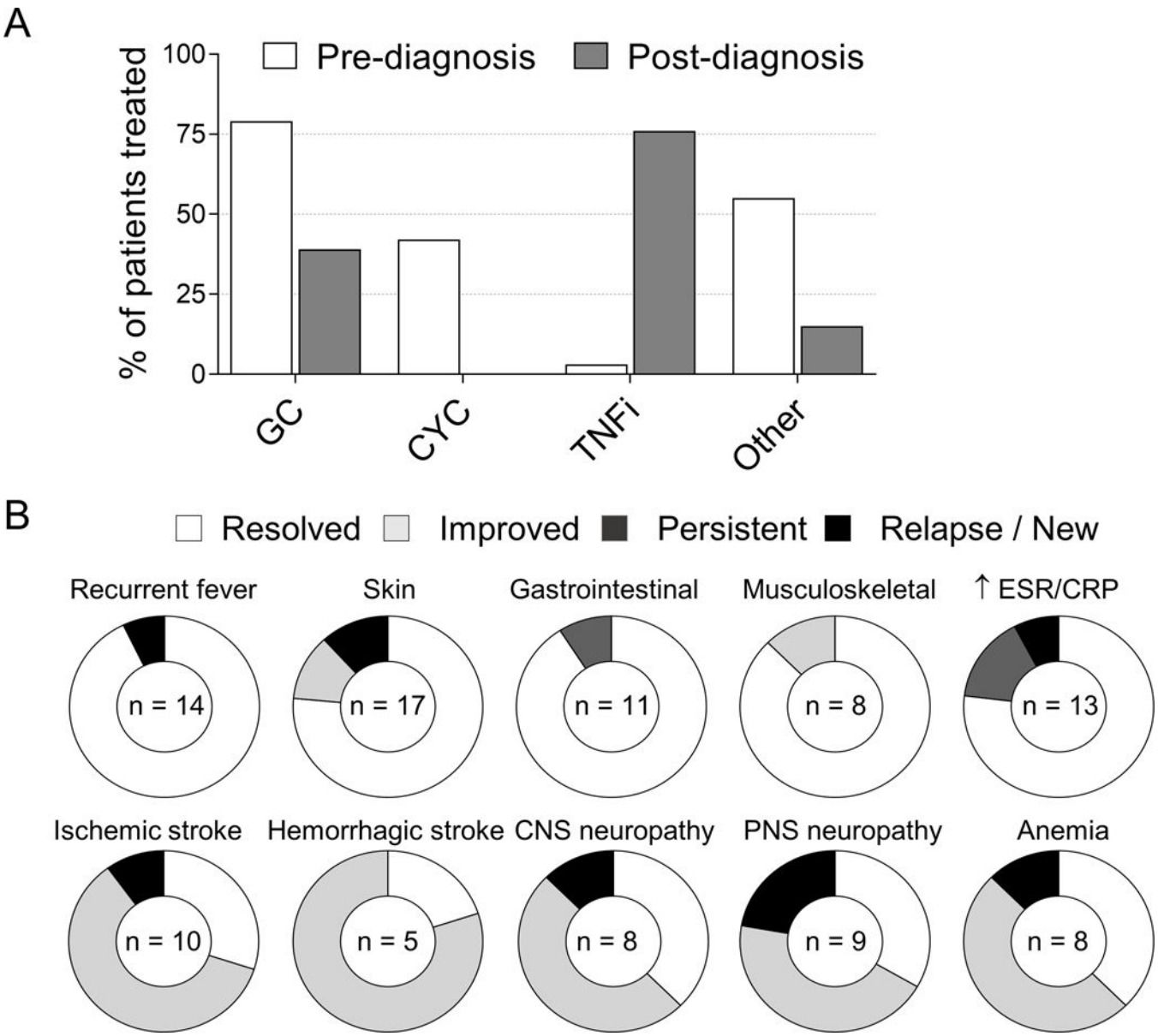

Figure 4. Therapeutic approaches and response to TNF inhibition in DADA2 patients.

A) Medication usage by patients before and after confirming the diagnosis of DADA2. B) Quantification of treatment responses in 21 patients maintained on TNFi. CRP: C-reactive protein; CNS: Central Nervous System; CYC: Cyclophosphamide; ESR: Erythrocyte Sedimentation Rate; GCs: Glucocorticoids; PNS: Peripheral Nervous System; TNFi: Tumor Necrosis Factor-α inhibitors. * Other drugs included azathioprine, cyclosporine A, intravenous immunoglobulin, methotrexate, mycophenolate mofetil, rituximab, sulfasalazine, thalidomide.

Table 1.

Summary of demographics and clinical features

Clinical feature	Over all (n=33)	Childhood onset (n=17)	Adult onset (n=16)	p value[#]
Age at onset (years, median, range)	15 (1-37)	8 (1-15)	25.5 (16-37)	-
Age at diagnosis (years, median, range)	23 (1-51)	16 (1-46)	32.5 (16-51)	-
Time to diagnosis (months, median, range)	52 (1-454)	101 (1-454)	33 (1- 290)	0.241
Males, n (%)	23 (69.7%)	11 (64.7%)	12 (75%)	0.708
Skin involvement, n (%)	24 (72.7%)	12 (70.6%)	12 (75%)	1.0
Neurological involvement, n (%)	26 (78.8%)	14 (82.4%)	12 (75%)	0.688
Central nervous system, n (%) **Stroke** **Ischemic** **Haemorrhagic** **Both**	22 (66.7%) 18 (54.5%) 9 (27.3%) 4 (12.1%) 5 (15.2%)	13 (76.5%) 9 (52.9%) 5 (29.4%) 2 (11.8%) 2 (11.8%)	9 (56.3%) 9 (56.3%) 4 (25%) 2 (12.5%) 3 (18.8%)	0.282
Peripheral nervous system, n (%)	13 (39.4%)	6 (35.3%)	7 (43.8%)	0.728
Constitutional symptoms, n (%)	18 (54.6%)	12 (70.6%	6 (37.5%)	**0037**
Gastrointestinal involvement, n (%)	15 (45.5%)	10 (58.8%)	5 (31.3%)	0.166
Renal involvement, n (%)	16 (48.5%)	9 (52.9%)	7 (43.8%)	0.732
Haematological involvement, n (%) **Anaemia** **Leukopenia**	10 (30.3%) 9 (27.3%) 1 (3%)	8 (47.1%) 7 (41.2%) 1 (5.9%)	2 (12.5%) 2 (12.5%) 0 (0%)	**0.031**
Other manifestations, n (%) **Arthritis / arthralgia** **Ocular involvement** **Central retinal artery occlusion** **Loss of peripheral pulses** **Testicular pain** * **Pregnancy loss** *	10 (30.3%) 6 (18.2%) 4 (12.1%) 3 (9.1%) 4/23 (17.4%) 2/ 10 (20%)	6 (35.3%) 5 (29.4%) 3 (17.7%) 2 (11.8%) 2 /11 (18.2%) NA	4 (25%) 1 (6.3%) 1 (6.3%) 1 (6.3%) 2 /12 (16.7%) 2 /4 (50%)	0.465 0.085 0.601 1.0 - -
Laboratory abnormality[@], n (%) **Elevated ESR** **Elevated CRP** **Positive ANA or ANCA** **	21/28 (75%) 20/22 (90.5%) 3/26 (11.5%)	10/16 (62.5%) 10/12 (83.3%) 1 /15 (6.7%)	11/12 (91.7%) 10/10 (100%) 2/11 (18.2%)	0.184 0.476 0.556

[#] p value for comparison between childhood onset and adult onset groups.

* Percentage is calculated only in the affected sex.

** ANA was tested in 26 patients and ANCA was tested in 22 patients.

[@] Percentage among patients with results.

ANA: Anti-nuclear antibody; ANCA: Anti-neutrophil cytoplasmic antibody; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; GI: Gastrointestinal; n: number; NA: Not applicable.