A cohort study on deficiency of ADA2 from China

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Abstract

Purpose: Deficiency of adenosine deaminase 2 (DADA2) is an autosomal recessive autoinflammatory disorder caused by biallelic loss-of-function mutations in ADA2, characterized by systemic inflammation, vasculitis, immunodeficiency, and/or hematologic abnormalities.

Methods: A retrospective analysis of patients with DADA2 diagnosed by whole exome sequencing at 17 rheumatology centers across China was conducted. Clinical characteristics, laboratory findings, genotype and treatment responses were analyzed.Results Thirty patients with DADA2 were enrolled in this cohort between January 2015 and December 2021. Median age at onset was 4.3 years and the median age at diagnosis was 7.8 years. All but one patient presented during childhood and two subjects in the cohort died from DADA2-related complications. The patients presented with systemic inflammation (92.9%), vasculitis (86.7%), and hypogammaglobinemia (73.3%) while one patient with pure red cell aplasia (PRCA) and bone marrow failure (BMF) with variable cytopenia was observed in our cohort. Twenty-three (76.7%) patients received TNF inhibitors and all achieved clinical remission. Homozygous variants and compound heterozygous variants were identified in 5 and 25 patients, respective. Two patients received hematopoietic stem cell transplantation (HSCT), and both got remission. Among 39 unique variants in our cohort, six is novel**.**

Conclusion: In order to establish the diagnosis early and prevent devastating clinical outcomes, genetic screening and/ or testing of adenosine deaminase enzymatic activity should be performed in patients with suspected clinical features. TNF inhibitors can be first line drug for patients with vasculitic DADA2. HSCT is an alternative choice for patients with refractory to TNF inhibitors or hematological disorders.

Introduction

Deficiency of adenosine deaminase 2 (DADA2, OMIM 165688) is an autosomal recessive autoinflammatory disease caused by biallelic loss-of-function mutations in the ADA2 gene, formerly named CECR1 (cat eye syndrome chromosome region, candidate 1), located at chromosome 22q11.1 [1–3]. It was first described in 2014 by two separate groups as a mimic of polyarteritis nodosa (PAN) and recurrent strokes [1, 2]. However, the clinical spectrum of the disease has expanded considerably since its initial description.

DADA2 is usually a childhood-onset disease, which presents with systemic inflammation, vasculitis, humoral immunodeficiency, and/or hematologic abnormalities [1, 2, 4–12]. Inflammatory features include recurrent fevers, weight loss, mild to moderate anemia, elevated erythrocyte sedimentation rate (ESR) and C-reactive protein. Vasculitis, which can involve in multiple organs, may manifest as rash (often livedo racemosa/reticularis), early-onset ischemic (lacunar) and/or hemorrhagic strokes, musculoskeletal involvement (arthralgia/ arthritis and/or myalgia/myositis). More severe involvement may lead to ischemic injury to the intestine, kidney, and/or digits or to progressive central neurologic deficits (ataxia, dysarthria, cranial nerve palsies, cognitive impairment) [11–17]. Hypertension and hepatosplenomegaly are often found [1, 2]. Patients may display humoral immunodeficiency of variable severity characterized by low immunoglobulin levels and increased risk of infection [1, 2, 4]. Hematologic disorders are present in some patients as pure red cell aplasia (PRCA) that mimics Diamond-Blackfan anemia, lymphopenia, neutropenia, thrombocytopenia, or pancytopenia and bone marrow failure (BMF)[5, 8, 18–22].

Adenosine deaminase 2 (ADA2) is an extracellular enzyme mainly secreted by myeloid cells, i.e., monocytes, macrophages, dendritic cells [15, 23, 24], and is a dimeric enzyme with four domains, including signal peptide, catalytic domain, putative receptor binding domain, and dimerization domain [10, 23, 25]. It is clear that biallelic homozygous or compound heterozygous mutations in the *ADA2* gene leading to decreased ADA2 activity are responsible for the disease [1, 2]. Missense variants are most common, but nonsense mutations, insertions/deletions (indels), splice-site mutations, copy number variation (CNV) and structural variation have been described [10, 15, 25–28].

Although DADA2 is rare, research on this disease can help to elucidate its pathogenesis. The long-term goal is to attain early diagnosis of DADA2 and to prevent devastating clinical outcomes. This study describes the clinical and genetic features in a Chinese cohort of 30 patients with DADA2 from 17 centers in China. We discuss novel clinical insights gained from the genetic findings in each case, summarize the current knowledge of DADA2, and suggest clinical features that may alert clinicians to suspect the disease.

Materials And Methods

Study design

This study was approved by ethics committees at Children's Hospital of Fudan University, Shanghai, China, and was designed as a retrospective cohort study. The inclusion criteria in this study include a compulsory criterion: biallelic mutations in the ADA2 gene, plus at least one of the followings: (1) systemic inflammation, (2) vasculitis, (3) humoral immunodeficiency, (4) hematologic abnormalities, (5) low levels of Adenosine deaminase 2 (ADA2) enzymatic activity. All patients were enrolled through a nationwide collaboration with approval by the local ethics committees. Research diagnostic testing was performed with written informed consent from the parent or themselves (more than 18 years). All the authors vouch for the accuracy and completeness of the data and analyses reported and for the fidelity of the study to the protocol.

Ada2 Activity Detection

We assayed ADA2 activity in the serum of all patients with novel ADA2 mutations and their parents using a commercial kit (Diazyme Laboratories) except P2, whose parents refuse ADA2 activity testing. Peripheral blood was taken from patients with ADA2 mutations and their parents. Serum was separated by centrifugation, total ADA activity was detected by an automatic biochemical analyzer, and ADA2 activity was detected by inhibiting ADA1 activity with EHNA. The parents and healthy controls were used as control.

DNA Sequencing,

Genomic DNA was extracted and purified from peripheral leukocytes in whole-blood samples by a DNA isolation kit (Qiagen, Hilden, Germany). Targeted exome capture was conducted on the genomic DNA from each patient by using the SureSelect Human All Exon Target Enrichment System (Agilent). The captured whole exomes were sequenced on the Illumina HiSeq 2500 Sequencer platform (Illumina, San Diego, CA, USA). Whole exome sequencing (WES) analysis and bioinformatic analysis was performed in patient's families as previously described [29]. Variants identified by WES analysis were confirmed by Sanger sequencing. The deleteriousness of the selected variants was subsequently predicted by various bioinformatics programs (SIFT, Polyphen2, PROVEAN, M-CAP, fathmm-MKL), and the variants were retained if their changes to the resulting proteins were damaging. All raw data of sequencing from 17 centers were analyzed.

Rna Sequencing

Libraries were prepared with one microgram RNA by using NEBNext Ultra RNA Library Prep Kit for Illumina(NEB) following manufacturer's recommendation. Prepared libraries were sequenced on Illumina Novaseq platform and 150-bp pair-end reads were generated. Reads were mapped to human genome(GRCh38) with STAR(v2.7.10).

Deletion Detection

Sequencing reads were visualized by using IGV tools. Reads were colored by red and blue based on read strand, respectively. Absence of coverage of patient's exon 7 and the abnormal transcript of patient both could be observed directly in IGV. The location on which the breakpoint of large deletion resided was detected by RepeatMasker(www.repeatmasker.org).

Statistical analysis

Statistical analyses were performed using the statistical package SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22 and Microsoft Excel (Microsoft Office 2016 version 16.0; Microsoft Corporation, Redmond, WA, USA). Continuous variables are summarized as the median and range, and categorical variables are presented as percentages and frequencies. The differences between two groups were analyzed by the Mann-Whitney U test, and chi-square test was used for comparison of categorical variables. A p value of ≤ 0.05 was considered significant.

Results

Clinical characteristics

Between January 2015 and December 2021, 30 patients (10 female and 20 male patients, respectively) from unrelated family were met the inclusion criteria in 17 centers in China. All but one subject were children. The median age at presentation was 4.3 years (range 9 days to 25.6 years), and median age at diagnosis was 7.8 years of age (range 1.0 to 30.0 years). Of these patients, recurrent fevers and rash was the first symptom in 23 patients, recurrent fevers in three patients, recurrent fever and respiratory tract infection in one patient. The skin and nervous system were the most commonly involved organs, affecting 86.7% and 60.0% of patients, respectively (Table 1). LR (60.0%) and nonspecific rash (60.0%) were common features in cutaneous involvement. 46.7% of the patient had experienced at least one ischemic stroke, and 20.0% hemorrhagic stroke. Hepatosplenomegaly was present in 23.3% of patients, splenomegaly in 16.7% of patients, and hepatomegaly in 3.3% of patients. Other common organ-specific features included those affecting joints (arthritis/arthralgia, 36.7%), gastrointestinal tract (16.7%), eye (Central retinal artery occlusion, 16.7%) and hypertention (10.0%). Less common organ involved was noted, such as muscle in two patients and heart in one patient. Systemic inflammation was noted in 93.3% of patients, which is defined as one of the followings, recurrent fever (90.0%), weight loss (33.3%), and elevated ESR (66.7%) or elevated CRP (86.7%). The prevalence of hypogammaglobinemia in our cohort is 73.3%. The frequency of low level of IgM, IgA and IgG is 66.7%, 33.3% and 26.7%, respectively.

Table 1
Clinical feature of 30 patients with ADA2 mutations

Characteristic	
Male gender, n (%)	20 (66.7)
Children, n (%)	29 (96.7)
Median age at onset(y)	4.3
Median age at diagnosis(y)	7.8
Death n (%)	2 (6.7)
Clinical features, n (%) or no./total no	
Systemic inflammation	28 (93.3)
Recurrent fevers	27 (90.0)
Weight loss	10 (33.3)
Vasculitis	26 (86.7)
Cutaneous involvement	26 (86.7)
Livedo racemosa/reticularis	18 (60.0)
Rash	18 (60.0)
Oral/skin Ulcer	5 (16.7)
Erythema nodosum / nodosum	4 (13.3)
Raynaud phenomenon	4 (13.3)
Digital gangrene	1 (3.3)
Arthritis/arthragia	11 (36.7)
Muscle involvement	2 (6.7%)
Nervous involvement	18 (60.0)
Ischemic stroke	14 (46.7)
Hemorrhagic stroke	6 (20.0)
Central nervous system	17 (56.7)
Headache	3 (10.0)
Peripheral neuropathy	1 (3.3)
Ophthalmological findings	5 (16.7)
Central retinal artery occlusion	5 (16.7)
Gastrointestinal involvement	5 (16.7)
Intestinal hemorrhage	2 (6.7)
Intestinal perforation	2 (6.7)
Intestinal necrosis	1 (3.3)
Splenomegaly	5(16.7)
Hepatomegaly	1 (3.3)
Hepatosplenomegaly	7 (23.3)
Heart involvement	1 (3.3)
Hypertention	3 (10.0)
Immunodeficiency	22 (73.3)
Hypogammaglobulinemia	22 (73.3)
Recurrent infection	4 (13.3)

Systemic inflammation is defined as one of the followings, recurrent fever, weight loss, elevated ESR, or elevated CRP; weight loss is defined as less than average weight of the same age and sex reduce two standard deviation(X-2SD).

Characteristic	
Hematologic abnormalities	22 (73.3)
Erythrocytopenia	22 (73.3)
Leukopenia/neutropenia	6 (20.0)
Lymphopenia	2 (6.7)
Pancytopenia	1 (3.3)
Laboratory findings, n (%) or no./total no.	
Elevated erythrocyte sedimentation rate	20 (66.7)
Elevated C-reactive protein	26 (86.7)
Decreased hemoglobin	22 (73.3)
Low level IgM	20 (66.7)
Low level IgA	10 (33.3)
Low level IgG	8 (26.7)
Low ADA2 activity	15/15
Positive ANA	3 (10.0)
Skin biopsy	6
Polyarteritis nodosa	3 (50.0)
Vasculitis	2 (33.3)
Panniculitis	1 (16.7)
Systemic inflammation is defined as one of the followings, recurrent fever, weight loss, elevated ESR, or elevated CRP; weight loss is defined as less than average weight of the same age and sex reduce two standard deviation(X-2SD).	

24 patients were diagnosed in department of rheumatology, four patients in department of neurology (three children, one adult), one patient (P28) in adult respiratory department, and one patient (P24) in department of hematology. All patients underwent thorough clinical and laboratory investigations in one of 17 centers, including genetic evaluation. The clinical and laboratory characteristics of the patients are provided in Table S1 and S2. Summarized data for the cohort are presented in Table 1. Consanguineous parents were noted in three families. 12 patients were reported before [30–32], and the others were first described in the cohort.

Novel Pathogenic Variants In Dada2

An average of 12.3 Gb of raw sequence data was generated with 94.38× depth of exome target regions for each individual as paired-end 150 base pair reads. 93.3% of the raw date sequencing quality was above Q30. The coverage of at least 10× and 20× of the target regions was 99.63% and 97.61%, respectively. All ADA2 variants were confirmed by Sanger sequencing and searched in databases, including gnomAD, ExAC, 1000 Genomes, HGMD, and infevers. Missense mutations were assessed for their potential to disrupt protein function via SIFT, Polyphen2, PROVEAN, M-CAP, fathmm-MKL (Table 2).

Table 2
Genetic information in patients with ADA2 mutations

Case	Genomic change[1]	cDNA change[2]	Amino acid Substitution[5]	Novel mutation[5]	Exon[5]	Mutation type	Mutation status	Mutation origin	Parental Consanguin
1	g.17181578T > C	c.1443-2A > G	-	-	Intron9	Splicing	Hom	Parents	Y
2	g.17209536C > T	c.142G > A	p.G48R	-	2	Missense	Het	Paternal	N
	g.17209357delT	c.321delA	p.A109Lfs*11	Novel	2	Frameshift	Het	Maternal	
3	g.17182626T > A	c.1217A > T	p.E406V	-	8	Missense	Het	Paternal	N
	g.17209578G > A	c.100C > T	p.R34W	-	2	Missense	Het	Maternal	
4	g.17209534delC	c.144delG	p.R49Gfs*4	-	2	Frameshift	Het	Maternal	Y
	g.17203738G > A	c.578C > T	p.P193L	-	4	Missense	Het	Paternal	
5	g.17209539C > G	c.139G > C	p.G47R	-	2	Missense	Hom	Parents	Y
6	g.17209539C > G	c.139G > C	p.G47R	-	2	Missense	Het	Maternal	N
	g.17188355G > T	c.1065C > A	p.F355L	-	7	Missense	Het	Paternal	
7	g.17156950_17215337del	-	-	-	-	Deletion	Het	Maternal	N
	g.17187845_17188621del	-	-	-	-	Deletion	Het	Paternal	
8	g.17209539C > A	c.139G > T	p.G47W	-	2	Missense	Het	Maternal	N
	g.17207129A > G	c.484T > C	p.W162R	Novel	3	Missense	Het	Paternal	
9	g.17191715A > C	c.849T > G	p.F283L	Novel	5	Missense	Hom	Parents	N
10	g.17189998G > A	c.916C > T	p.R306X	-	6	Nonsense	Het	Paternal	N
	g.17188351C > T	c.1069G > A	p.A357T	-	7	Missense	Het	Maternal	
11	g.17181904T > C	c.1358A > G	p.Y453C	-	9	Missense	Hom	Parents	N
12	g.17203745delG	c.571delC	p.Q191Sfs*5	-	4	Frameshift	Hom	Parents	N
13	g.17181925A > G	c.1337T > C	p.F446S	-	9	Missense	Het	Paternal	Y
	g.17182022C > T	c.1240G > A	p.V414M	-	9	Missense	Het	Maternal	
14	g.17209536C > T	c.142G > A	p.G48R	-	2	Missense	Het	Maternal	N
	g.17188348C > T	c.1072G > A	p.G358R	-	7	Missense	Het	Paternal	
15	g.17207233T > A	c.380A > T	p.N127I	-	3	Missense	Het	Paternal	N
	g.17188443C > A	c.977G > T	p.G326V	-	7	Missense	Het	Maternal	
16	g.17188416T > G	c.1004A > C	p.H335P	-	7	Missense	Het	Paternal	N
	g.17207224T > C	c.389A > G	p.Y130C	-	3	Missense	Het	Maternal	
17	g.17209539C > G	c.139G > C	p.G47R	-	1	Missense	Het	Paternal	N
	-	Exon 7	-	-	7	Deletion	Het	Maternal	
18	g.17207107C > T	c.506G > A	p.R169Q	-	3	Missense	Het	Paternal	N
	g.17207220delC	c.393delG	p.R131Sfs*53	-	3	Frameshift	Het	Maternal	
19	g.17181904T > C	c.1358A > G	p.Y453C	-	9	Missense	Het	Paternal	N
	g.17182734T > A	c.1109A > T	p.N370I	-	8	Missense	Het	Maternal	
20	g.17209536C > T	c.142G > A	p.G48R	-	2	Missense	Het	Paternal	N
	g.17207108G > C	c.505C > G	p.R169G	-	3	Missense	Het	Maternal	

1. **gDNA reference: NC_000022**

2. **cDNA reference: NM_001282225**

3. **Computational prediction: Letters are on behalf of the prediction of SIFT, Polyphen2, PROVEAN, M-CAP and fathmm-MKL in turn. D: damage; B: Benign; T: possibly damaging; dot: no prediction**

4. **Y: yes; N: no.**

5. **-: NA**

Case	Genomic change[1]	cDNA change[2]	Amino acid Substitution[5]	Novel mutation[5]	Exon[5]	Mutation type	Mutation status	Mutation origin	Parental Consanguin
21	g.17209536C > T	c.142G > A	p.G48R	-	2	Missense	Het	Paternal	N
	g.17207108G > C	c.505C > G	p.R169G	-	3	Missense	Het	Maternal	
22	g.17209534_17209535delinsA	c.143_144delinsT	p.G48Vfs*5	-	2	Frameshift	Het	Paternal	N
	g.17209581dupT	c.97dupA	p.T33Nfs*29	-	2	Frameshift	Het	Maternal	
23	g.17209581dupT	c.97dupA	p.T33Nfs*29	-	2	Frameshift	Het	Maternal	Y
	g.17188348C > T	c.1072G > A	p.G358R	-	7	Missense	Het	Paternal	
24	g.17181955A > G	c.1307T > C	p.M436T	Novel	9	Missense	Het	Paternal	N
	g.17181928A > G	c.1334T > C	p.M445T	Novel	9	Missense	Het	Maternal	
25	g.17209539C > A	c.139G > T	p.G47W	-	2	Missense	Het	Paternal	N
	g.17207108G > C	c.505C > G	p.R169G	-	3	Missense	Het	Maternal	
26	g.17209538C > A	c.140G > T	p.G47V	-	2	Missense	Het	Maternal	N
	g.17182768A > T	c.1082-7T > A	-	-	Intron7	Splicing	Het	Paternal	
27	g.17181904T > C	c.1358A > G	p.Y453C	-	8	Missense	Het	Paternal	N
	g.17182768A > T	c.1082-7T > A	-	-	Intron7	Splicing	Het	Maternal	
28	g.17209539C > A	c.139G > T	p.G47W	-	2	Missense	Hom	Parents	Y
29	g.17209581dupT	c.97dupA	p.T33Nfs*29	-	2	Frameshift	Het	Paternal	N
	g.17207267delT	c.346delA	p.I116Lfs*4	Novel	3	Frameshift	Het	Maternal	
30	g.17189980G > A	c.934C > T	p.R312X	-	6	Nonsense	Het	Paternal	N
	g.17209400A > G	c.278T > C	p. I93T	-	2	Missense	Het	Maternal	N

1. **gDNA reference: NC_000022**

2. **cDNA reference: NM_001282225**

3. **Computational prediction: Letters are on behalf of the prediction of SIFT, Polyphen2, PROVEAN, M-CAP and fathmm-MKL in turn. D: damage; B: Benign; T: possibly damaging; dot: no prediction**

4. **Y: yes; N: no.**

5. **-: NA**

Among the 30 patients, 24 patients possessed compound heterozygous variants while six were found having homozygous variants in the ADA2 (Table 2). One patient carried two deletions (g.17156950_17215337del and g.17187845_17188621del), which were derived from his mother and father, respectively (Table 2 and Fig. 1a, b, c, d). These two deletions were detected by visualizing the raw data of RNA-seq and WES using IGV (Supplementary Fig. 1). The larger deletion is a 58kb deletion including HDHD5 exon1 to ADA2 exon2 (Fig. 1e) and the smaller deletion includes ADA2 exon7 (Fig. 1f). The mechanism of both deletions is due to Alu-mediated deletion.

In total, there are six novel ADA2 variants in our cohort (Table 2). Plasma or serum ADA2 activity of patients carrying novel ADA2 variants are tested. All of these patients exhibit obviously lower ADA2 activity comparing with healthy and carrier control (Fig. 2a). We also transfect plasmid of ADA2 mutants with novel variants into HEK293T cells and test ADA2 activity in their supernatants and cell lysates of cell culture. Expression level of these ADA2 mutants is shown (Fig. 2d). ADA2 activity of these ADA2 mutants in whole cell lysates (Fig. 2b) and supernatants of cell culture (Fig. 2c) are both lower than wild-type ADA2. Therefore, these novel ADA2 variants are pathogenic variants.

Treatment and outcomes.

The median follow-up duration was 20.2 months (range, 5 to 36 months) after the diagnosis of DADA2. Two patient died from macrophage activation syndrome (MAS), characterized by fever, splenomegaly, multilineagecytopenia, hyperferritinemia, hypertriglyceridemia, and hypofibrinogenemia. Before the diagnosis of DADA2, 27(90%) patients were treated with high-dose glucocorticoids, and 6 patients received non-steroidal anti-inflammatory drugs (NSAIDs) (Table 3 and Table S3). In total, 26(86.7%) patients received one or more traditional DMARDs, such as methotrexate (n = 7, 23.3%), cyclosporine (n = 5, 16.7%), mycophenolate mofetil (n = 5, 16.7%), cyclophosphamide (n = 4, 13.3%), hydroxychloroquine (n = 3, 10.0%), Sulfasalazine (n = 1, 3.3%). eleven patients (36.7%) received IVIG, and five were given thalidomide. In addition, four were treated with tocilizumab. Except thalidomide, the overall efficacy of these interventions was suboptimal. The patients continued to have either persistent disease or relapse. After the diagnosis of DADA2 by sequencing and/ or ADA2 activity detection, 23 patients with active disease received TNF inhibitors, including etanercept (n = 13), infliximab (n = 1), adalimumab (n = 9). TNF inhibitor was effective in controlling the fever episodes, vasculitis, and prevention of strokes in patients during follow-up. Two patients accepted hematopoietic stem cell transplantation (HSCT). One patient reported before manifest as recurrent fever and rashes from the age of 3 months, developed dry gangrene of the fingers at

5 months of age [31]. She was treated with glucocorticoid and tocilizumab with poor response. She further developed right central retinal artery occlusion. Due to the lack of response to treatment and severe organ involvement, HSCT was performed. The other patient with PRCA and BMF with variable cytopenia was no response to high- dose glucocorticoids and cyclosporine, and accepted HSCT. Two patients both are currently in remission. Thalidomide treated in three patients was also effective in controlling the fever episodes and vasculitis. Of these three patient, two only received thalidomide, one was treated with thalidomide combined with adalimumab (Table S3). The other two patients with thalidomide got partial remission (Table S3).

Table 3
Therapy for patients with DADA2

Therapy	Medicine	Patient, n(%)	The efficacy
Traditional DMARDs	Sulfasalazine	1 (3.3)	Little effect
	Methotrexate	7 (23.3)	Little effect
	Hydroxychloroquine	3 (10.0)	Little effect
	Cyclophosphamide	4 (13.3)	Little effect
	Tacrolimus	1 (3.3)	Little effect
	Cyclosporine	5 (16.7)	Little effect
	Mycophenolate	5 (16.7)	Little effect
Biological DMARDs	Tocilizumab	4 (13.3)	Failure to control inflammation
	Etanercept	13 (43.3)	Controlling the fever episodes, vasculopathy, and prevention of strokes
	Infliximab	1 (3.3)	
	Adalimumab	9 (30.0)	
Others	Glucocorticoid	27 (90.0)	Little effect
	IVIG	11 (36.7)	Little effect
	NSAIDs	6 (20.0)	Little effect
	Thalidomide	5 (16.7)	Controlling the fever episodes, vasculopathy, and prevention of strokes
	HSCT	2 (6.7)	Control both the immunological, the hematological, and the vascular phenotype of DADA2

DMARDs: disease-modifying anti-rheumatic drugs; IVIG: intravenous immunoglobulin; HSCT: Hematopoietic stem cell transplantation; NSAIDs: nonsteroidal antiinflammatory drugs.

Discussion

In this report, we describe the clinical features, laboratory findings, genotypes, and treatment responses in 30 Chinese patients with DADA2. This constitutes the largest cohort study on DADA2 from China to date. The mortality of DADA2 in our cohort is 6.7%, similar to another study (8.4%) [33–38]. The skin and nervous system were the most commonly involved organs. LR and nonspecific rash were common features in cutaneous involvement. Vasculitis involved almost all organs was identified in all patients, which also confirms that DADA2 is a systemic disease.

Systemic inflammation was noted in 93.3% of patients. CRP is the more sensitive index for inflammation compared to ESR. DADA2 is now a well-recognized mimic of PAN with systemic inflammation and vasculitis features [1, 2, 10, 15, 25, 27, 37]. Biallelic mutations in ADA2 gene were identified in 25–31% of childhood PAN [7, 36–38]. Therefore, genetic testing and/or ADA2 activity detection should be considered in patients with recurrent fever accompanied unexplained elevated ESR and/or CRP, and in patients with recurrent fever and livedo racemosa/reticularis or rash, especially in children with PAN-like vasculitis.

The prevalence of hypogammaglobinemia in our cohort is 73.3%, which is similar to the general estimate for 13 cohorts range from 40–100% [1, 2, 5, 7, .9, 11, 33–37]. The prevalence of hypogammaglobinemia is high, but recurrent infection or severe infection was not noted in all patients with hypogammaglobinemia in other studies [1, 5, 34, 36], so is our cohort. There seven patients with recurrent infections in our cohort, which are respiratory tract infection, and are easy to be controlled by antibiotics. Interestingly, one patients (P28) only had recurrent fever and respiratory tract infection without LR, rash and other organ involvement after age of 15. He was diagnosed with immune deficiency disease because of low level of IgM, IgA and IgG, and diagnosed with DADA2 by WES until adulthood. Now, more and more studies reveal mild humoral immunodeficiency with low immunoglobulin levels represents a common clinical feature of DADA2 regardless of the presenting phenotype [10, 27, 38]. Therefore, patients with antibody deficiencies should also be considered to screen gene testing.

Hematologic abnormalities are primary presentation in some patients, even without vasculitis or systemic inflammation [5, 8]. PRCA and BMF with variable cytopenia was observed in one patient in our cohorts, without vasculitis and systemic inflammation. Mild to moderate anemia and leukopenia/neutropenia was noted in 73.0% and 20% of patients, respectively. Frequency of anemia is consistent with that of recurrent fever (90.0%), and it may be caused by recurrent chronic systemic inflammation.

Patients in our cohort show no response to glucocorticoid, NSAIDs, IVIG, and traditional DMARDs. TNF inhibitors were given to 23 patients with disease flare. Our study revealed TNF inhibitors have the effect of controlling the fever episodes and vasculitis in 23 patients. In addition, all patients treated with TNF inhibitors do not develop stroke or relapse during follow-up duration, which ranges from 5 to 36 months. Three patients received thalidomide, and clinical symptoms were also improved significantly. HSCT is an alternative choice for patients with refractory to TNF inhibitors or hematological disorders, which not only rescues the hematological and immunological phenotype, but also vascular phenotype in DADA2[8, 9, 37, 42–45].

In total, 39 variants were detected in our cohort. Of these variants, six variants are novel (Table 2), which expands the spectrum of known mutations in ADA2. Mutations detected in our cohort are in each coding exon of the ADA2 gene, and involved in four domains of protein encoded by ADA2. None hot spots were found in our cohort. The most common variant is the mutation p.T33Nfs*29 identified in four patients, which located in dimerization domain. The p.G47R mutation frequently seen in Georgian, Jewish and Turkish, the p.R169Q variant mainly noted in the Netherlands, Belgium and Finland, and the p.T360A mutation more common in Italy were all observed in our cohort [2, 7, 34, 46].

Currently, the diagnosis of DADA2 is established in a proband with suggestive clinical and laboratory findings. Most patients with DADA2 from all over the world are from department of rheumatology in different centers [1, 2, 5, 7, .9, 11, 33–37, 47, 48]. Patients with haematological abnormalities, or humoral immunodeficiency, and/or early stroke may likely to be misdiagnosed. Early diagnosis of ADAD2 is important as it provides the chance of a timely treatment to improve the patient outcome and as the attainment of an early response dampens the disease course by minimizing organ damage. Therefore, we propose flow chart of diagnosis of DADA2 in Fig. 2. Molecular diagnosis should be performed in patients with at least one of followings, unexplained reason of systemic inflammation, or vasculitis, or humoral immunodeficiency, or severe hematologic abnormalities, especially in children.

Limitation

Most patients of this study are from rheumatology department, and some patients with hematologic abnormalities, stroke and recurrent infection as the first symptoms may be not included. It is necessary to improve the understanding of DADA2 among pediatric neurology, hematology and immunology specialists in China.

Conclusion

This study includes the largest number of Chinese DADA2 patients to date, and identified six novel ADA2 pathogenic variants. DADA2, a heterogeneous disease, is characterized by systemic inflammation, vasculitis, immunodeficiency and hematologic disorders. In order to diagnosis early and prevent devastating clinical outcomes, gene screening and/or ADA2 activity detection should be performed in patients with one of the followings: unexplained reason of systemic inflammation, or vasculitis, or humoral immunodeficiency, or severe hematologic abnormalities, especially in children. Early diagnosis of DADA2 is important, because it can improve the patient outcome and prevent devastating complications. TNF inhibitors can be first line drug for patients with vasculitic DADA2. HSCT is an alternative choice for patients with refractory to TNF inhibitors or hematological disorders.

Declarations

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Data availability statement The original contributions generated for the study are included in the article and Supplementary Material. Data in this article is available on GSA-Human (https://ngdc.cncb.ac.cn/gsa-human), accession number: HRA001673.

Author contributions G-mL, XH, WW, H-xT, M-pL, X-mT, YL, FD, JY, X-nW, C-cL, S-hW, W-jZ, YW and B-bW performed the experiments. FZh, H-mL, W-zG, RF, T-nZ, YW, LG, W-jT, HC, Q-yZ, X-zL, J-gL, P-fT, T-jJ, Z-xZ, S-rY, K-kY, HX, YW, JZ, HL, LZ, YZ and H-mS analyzed the data. G-mL and XH wrote the manuscript. QZ and LS conceived and supervised the project. All authors contributed to the article and approved the submitted version.

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Ethics statement This study was approved by ethics committees at Children's Hospital of Fudan University, Shanghai, China.

Consent to Participate written informed consent was obtained from patients' parents.

Consent for Publication Written informed consent was obtained from patients' parent.

Conflict of Interest The authors declare no competing interests.

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Figures

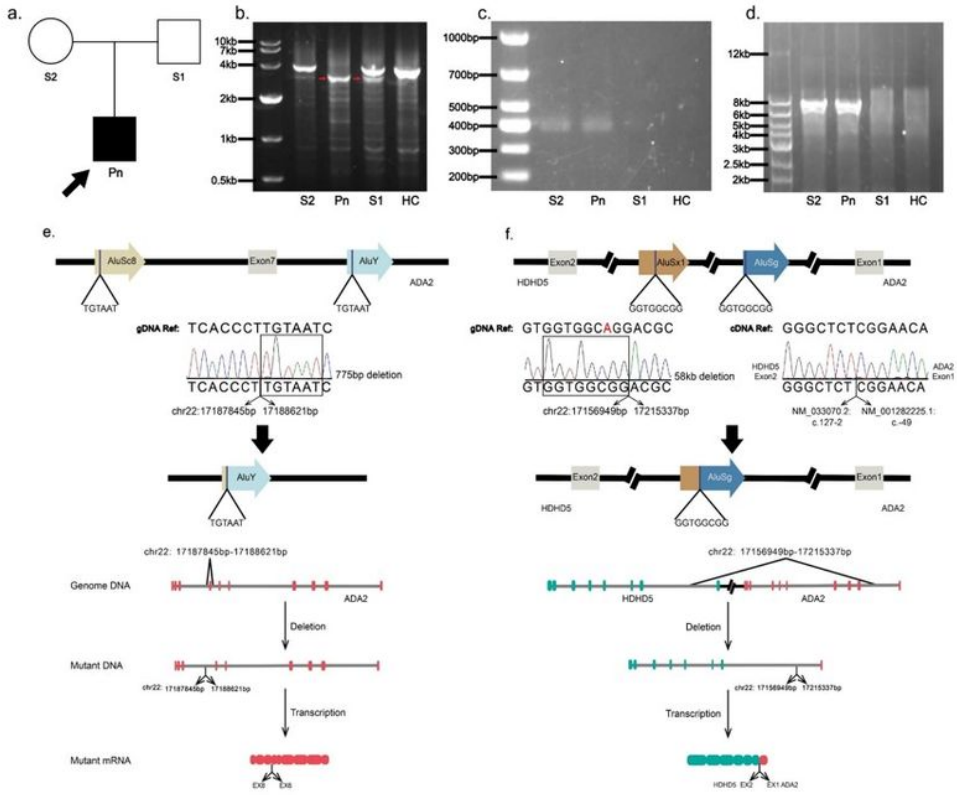

Figure. 1 (a) Pedigree of patient's family. **(b)** PCR using patient's gDNA with a set of primer flanking ADA2 exon 7 shows a homozygous deletion and a heterozygous deletion in Pn and S1, respectively. **(c)** PCR using patient's cDNA with a set of primer flanking a region from HDHD5 exon2 to ADA2 exon1, implicating a fusion transcript. **(d)** Long-range PCR using patient's gDNA with a set of primer flanking a region from HDHD5 exon1 to ADA2 exon2, indicating a deletion of about 58kb in Pn and S2. Schematic demonstration of Alu-mediated deletion of exon7**(e)** and of a long region from HDHD5 exon1 to ADA2 exon2**(f)**.

Figure 1

See image above for figure legend.

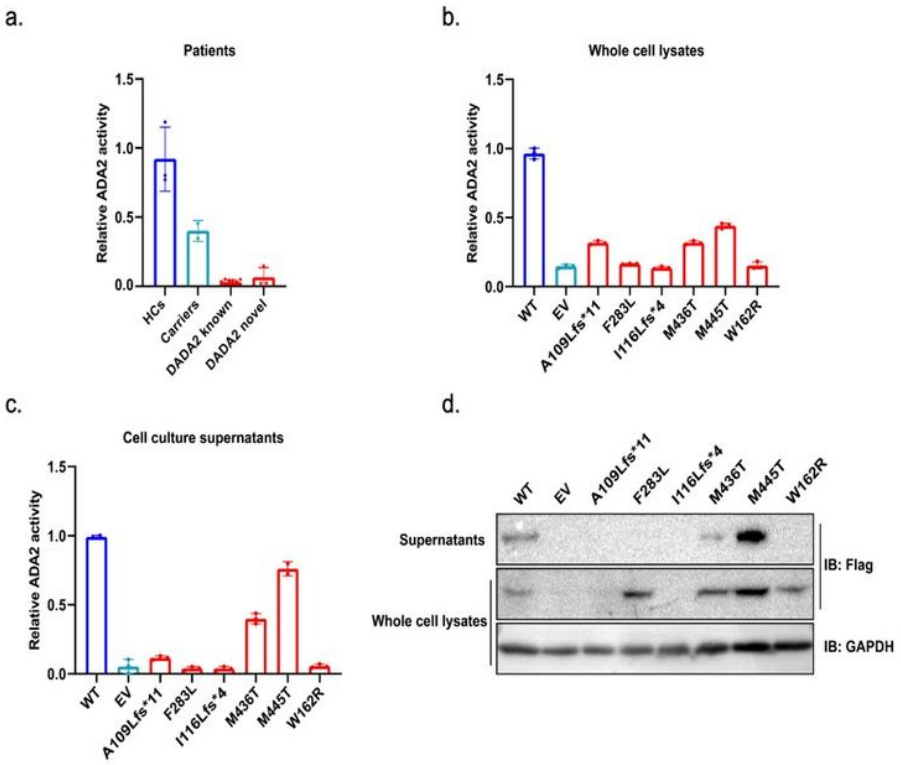

Figure. 2 ADA2 activity of **(a)** DADA2 patients possessing novel or known ADA2 pathogenic variants, **(b)** whole cell lysates and **(c)** supernatants of 293T cells transfected with ADA2 mutants is tested. **(d)** Western blots of 293T cells transfect with ADA2 mutants. As P9 has gone and P2 is lost to follow-up, ADA2 activity of these patients is not tested. HC, healthy control; WT, wild type; EV, empty vector.

Figure 2

See image above for figure legend.

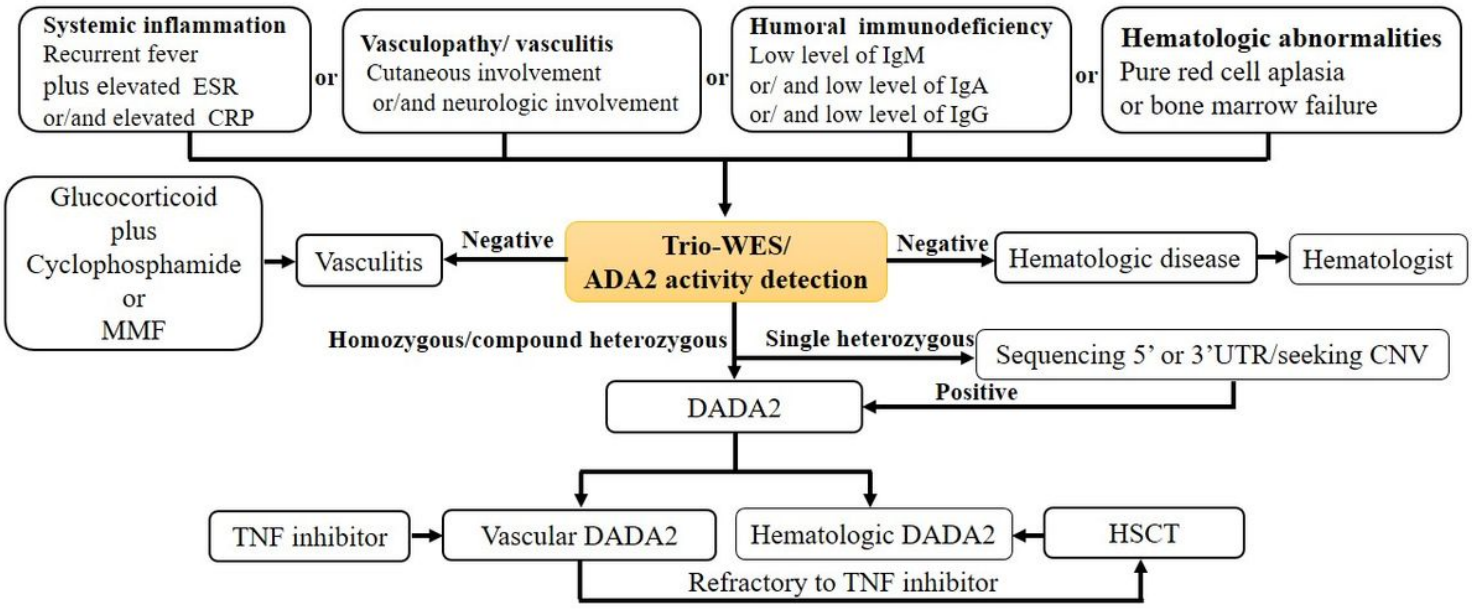

Figure.3 Flow chart of diagnosis and treatment of DADA2. HSCT: hematopoietic stem cell transplantation; MMF: mycophenolate mofetil;

Figure 3

See image above for figure legend.

Supplementary Files

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