







Chi-square test $p < 0.0001$

Genotype and functional correlates of disease phenotype in Deficiency of Adenosine Deaminase 2 (DADA2)

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Supplemental Methods

Literature review and case selection: We performed a comprehensive literature review of DADA2 case series and case reports published between February 2014 to July 2019. The PubMed database was queried using the search terms “DADA2”, “deficiency of adenosine deaminase 2” or “adenosine deaminase 2.” A total of 186 cases were reviewed and assigned to categories of vasculitis, PRCA, BMF or other phenotypes (including lymphoproliferation and asymptomatic patients). Cases selected from each publication and their phenotype are detailed in Table E4. Duplicate cases that appeared in multiple publications were analyzed only once. Cases with other phenotypes or incomplete data on *ADA2* mutations were excluded.

The vasculitis phenotype was defined by any clinical or biopsy-proven diagnosis of polyarteritis nodosa (PAN), cutaneous vasculitis, ischemic stroke, hemorrhagic stroke, or vasculitis of visceral organs. Applying these criteria to 11 major case series from around the world, we identified 100 cases of DADA2 with vasculitis as the predominant phenotype. Because the number of vasculitis cases is disproportionately higher than other phenotypes in existing case series, case reports of patients with vasculitis were not included.

Whereas primary hematologic presentations are less common, we compiled all cases with PRCA and BMF from the literature search. The PRCA group (n = 33) comprised of patients with severe anemia as the presenting features of DADA2, with minimal impact on other cell lineages. The BMF phenotype (n = 19) included cases with initial presentation of severe leukopenia, neutropenia, lymphopenia and/or thrombocytopenia. A complete list of mutations from the selected cases are displayed in Table E5.

Diagnostic testing for DADA2: Biallelic mutations in *ADA2* were confirmed for each patient by DNA sequencing (targeted Sanger sequencing, n = 1; next generation sequencing panels, n = 7; whole exome sequencing, n = 7). Plasma *ADA2* enzyme activity was confirmed as low in 7 patients.

Supplemental Figure Legends

Figure E1. Age of symptom onset stratified by disease phenotype. Scatter dot plot display of age of symptom onset for DADA2 patients stratified by disease phenotype. All cases from the current cohort and those selected from literature review were included. Median and interquartile range are displayed.

Figure E2. Functional and in silico analysis of missense *ADA2* mutations. A) Residual *ADA2* enzymatic activity of missense mutants grouped by disease phenotype. B-E) Predicted pathogenicity of *ADA2* missense variants by in silico algorithms including B) SIFT, C) Polyphen2, D) MutationTaster and E) Combined Annotation Dependent Depletion (CADD) score.

Figure E3. Distribution of DADA2 cases by genotype categories. Bar graph illustration of DADA2 cases stratified by disease phenotype and genotype category.

Table E1. Clinical characteristics of DADA2 patients with PRCA or BMF

ID	Onset (yr)	Sex	Mutations	Phenotype	Stroke	Skin vasculitis	HSM	GI	Recurrent infection	Treatment	Response to TNFi	Alive	
A-1	0.3	M	R49Afs*13 / R49Afs*13	PRCA	No	No	No	-	No	-	transfusion, CS	-	Yes
A-2	0.5	F	R49Afs*13 / R49Afs*13	PRCA	No	No	No	-	No	-	transfusion, AD, tacrolimus	No	Yes
A-3	0.1	F	R49Afs*13 / R49Afs*13	PRCA	No	No	No	-	No	-	transfusion, AD	No	Yes
B-1	12	M	G321E / G321E	PRCA	No	No	Yes	oral ulcer, colitis	No	-	Epo, CS, transfusions, HSCT	-	No
C-1	0.3	M	G358R / G358R	PRCA	Yes	No	Yes	-	No	-	transfusions, CS, ET	Yes	Yes
D-1	0.8	M	F178S / F178S	BMF	No	Yes	Yes	-	No	-	transfusion, IVIG, CS, MMF	-	Yes
E-1	12	F	K466Tfs*2 / K466Tfs*2	BMF	No	No	Yes	-	Yes	pneumonia, anal abscess, sepsis, necrotizing fasciitis	RTX, IVIG, CS, GCSF	-	No
F-1	4	F	R49Afs*13 / R49Afs*13	BMF	No	No	Yes	gingivitis	Yes	pneumonia, URI, UTI	GCSF, AD	No	Yes
G-1	3	M	G47W / G47W	BMF	No	No	Yes	colitis	Yes	necrotizing colitis, sepsis	Anakinra, MMF, sirolimus, AZA, RTX, GCSF, AD	No *	No
H-1	1.3	F	G358R / G358R	BMF	No	Yes	Yes	oral ulcer	Yes	Sinusitis, pneumonia	CS, ET, AD	Partial †	Yes
I-1	13	M	F212Del / V458D	BMF	No	No	Yes	oral ulcer	Yes	pneumonia, otitis media, sepsis	GCSF, ET, HSCT	No	No
J-1	0.1	F	G358R / G358R	BMF	No	No	Yes	gingivitis	Yes	endocarditis, sepsis	GCSF, CS, AD	No **	No
J-2	0.4	M	G358R / G358R	BMF	No	No	Yes	gingivitis, anal fistula	Yes	sepsis, fungal sinusitis	GCSF, CS, HSCT	-	No
K-1	10	F	F178S / F178S	BMF	No	No	No	gingivitis, oral ulcer colitis	No	-	CS, infliximab, methotrexate	Partial ††	Yes †
L-1	0.4	M	K449Nfs*2 / K449Nfs*2	BMF	No	No	Yes	Oral ulcer, gingivitis	Yes	otitis media, pneumonia, cellulitis	ET, CS, GCSF	Partial ††	Yes †

Abbreviations: AD, adalimumab; AZA, azathioprine; CS, corticosteroids; ET, etanercept; GCSF, granulocyte colony stimulating factor; Epo, erythropoietin; HSCT, hematopoietic stem cell transplant; HSM, hepatosplenomegaly; IVIG, intravenous immunoglobulins; MMF, mycophenolate mofetil; RTX, rituximab; URI, upper respiratory tract infection; UTI, urinary tract infection; * Patient received two doses of AD prior to death. ** Patient J-1 developed sepsis shortly after initiation of TNF inhibitor. † Patient H-1 showed improvement of fever and skin rash but cytopenia did not resolve. †† Patient K-1 and L-1 showed improvement of fever, oral ulcer and gingivitis but cytopenia remained the same. † Patients K-1 and L-1 each has a sibling who died from severe infection.

Table E2. Laboratory findings of DADA2 patients with PRCA or BMF

ID	Phenotype	Hgb (g/dL)	Retic (%)	MCV	WBC (10 ⁹ /L)	ANC (10 ⁹ /L)	ALC (10 ⁹ /L)	PLT (10 ⁹ /L)	CD4+ T (10 ⁶ /L)	CD8+ T (10 ⁶ /L)	CD19+ (10 ⁶ /L)	CD56+ (10 ⁶ /L)	IgG (mg/dL)	IgM (mg/dL)	IgA (mg/dL)
A-1	PRCA	4.7	0.30	68.7	7.5	825	5.9	481	1746	1587	1029	327	871	28	104
A-2	PRCA	7.1	0.30	83.7	11.8	5.7	5.8	442	1769	1235	1893	196	934	43	13
A-3	PRCA	4.9	0.30	86.7	13.1	3.7	8	337	3006	1142	3107	397	438	30	10
B-1	PRCA	6.8	0.10	91	3.8	2	1.2	252	249	165	42	28	660	178	25
C-1	PRCA	3.3	0.29	62	12.6	2.9	8.8	300	986	427	1127	100	303	73	11
D-1	BMF	6	0.30	90.4	3.2	1	2.1	41	1000	763	84	700	651	44	41
E-1	BMF	10.9	0.10	78.1	1	0.17	0.81	424	219	293	0	6	130	22.6	22.6
F-1	BMF	10.4	2.20	72.5	1.57	0.33	0.7	163	174	424	76	10	735	30	66
G-1	BMF	7.7	0.04	89.6	0.42	0.03	0.37	14	70	170	0	0	1330	33	70
H-1	BMF	12.1	0.8	74	2.1	1.26	0.4	114	336	59	28	112	487	12	28
I-1	BMF	11.6	1.50	75.8	2.01	0.24	1.38	180	365	262	31	64	274	6	33
J-1	BMF	8	2.00	75	5	0.05	5	250	2512	1507	2679	586	2040	767	69
J-2	BMF	9.8	2.40	69	5	0.05	4	600	1980	1584	220	484	1130	24	68
K-1	BMF	6.9	1.50	65	2	0.3	3	250	621	621	336	251	1010	89	163
L-1	BMF	9.5	2.60	75	7	0.3	6.5	250	735	8188	315	2730	1290	76	365

Abbreviations: Hgb, hemoglobin; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; MCV, mean corpuscular volume; Retic, reticulocyte.

Table E3. Autoantibody profiles in DADA2 patients with PRCA or BMF

ID	Phenotype	DAT	ANA	ANCA	Anti-dsDNA	Anti-ENA	Anti-cardiolipin	Anti-β2GP	Anti-neutrophil	Anti-platelet
A-1	PRCA	Neg								
A-2	PRCA	Neg								
A-3	PRCA	Neg								
B-1	PRCA	Neg	Neg				Neg			
C-1	PRCA	Neg	Neg							
D-1	BMF	Neg								
E-1	BMF		+ 1:100		Neg					
F-1	BMF		Neg	+ c-ANCA*	Neg					
G-1	BMF		Neg	Neg		Neg			Neg	
H-1	BMF		Neg				Neg	Neg	Neg	
I-1	BMF	Neg			Neg				Neg	Neg
J-1	BMF	Neg	Neg	Neg	Neg				Neg	
J-2	BMF									
K-1	BMF	Neg	Neg				Neg	Neg		
L-1	BMF	Neg	+ 1:160						+	

* Proteinase-3 and myeloperoxidase specific antibody testing were negative

Abbreviations: ANA: anti-nuclear antibodies; ANCA: anti-neutrophil cytoplasmic antibodies; β2GP: β2 glycoprotein; DAT: direct antiglobulin test; dsDNA: double-stranded DNA; ENA: extractable nuclear antigens; Neg: negative.

Table E4. Selection of cases from literature review

Authors	PMID#	Total	Vasculitis	PRCA	BMF	Excluded (reason*)
Alsultan et al.	29271561	1	0	0	1	0
Barzaghi et al.	30692987	1	0	0	1	0
Batu et al.	26233953	6	3	0	0	3 (duplicate)
Ben-Ami et al.	27514238	5	0	4	1	0
Caorsi et al.	28522451	15	14	0	0	1 (other phenotype)
Cipe et al.	29564582	1	0	0	1	0
Claassen et al.	30559313	1	0	1	0	0
Ghurye et al.	30924144	2	0	0	2	0
Gibson et al.	31008556	9	8	0	0	1 (partial genotype)
Hashem et al.	28974505	14	0	3	5	6 (duplicate)
Hashem et al.	28230570	1	0	1	0	0
Hsu et al.	27130863	1	0	0	1	0
Michniacki et al.	29411230	2	0	0	2	0
Nanthapaisal et al.	27059682	15	10	0	0	5 (other phenotype)
Navon-Elkan et al.	24552285	24	20	0	0	4 (2-partial genotype; 2-other phenotype)
Neishabury et al.	31097629	2	0	2	0	0
Ozen et al.	31043544	24	6	10	0	8 (2-partial genotype ; 6-duplicate)
Rama et al.	29681619	13	13	0	0	0
Sahin et al.	28516235	8	6	0	0	2 (Duplicate)
Sasa et al.	n/a *	2	0	2	0	0
Sundin et al.	29620681	1	0	0	1	0
Trotta et al.	29391253	9	3	0	3	3 (other phenotype)
Ulirsch et al.	30503522	9	0	9	0	0
Van Eyck et al.	25457153	2	0	0	1	1 (other phenotype)
Von Montfrans et al.	26867732	9	8	1	0	0
Zhou et al.	24552284	9	9	0	0	0
Total cases reviewed		186	100	33	19	34
Lee et al. (current)		15	0	5	10	
All cases combined		201	100	38	29	

* Duplicated cases were each analyzed only once. Partial genotype refers to patients with only one identified mutation. Other phenotypes include patients without frank features of vasculitis or hematologic abnormalities, asymptomatic individuals, and patients with primarily lymphoproliferative disease without features of other phenotypes.

** Pubmed ID not available. Abstract reference: Sasa et al. *Blood* 2015 126:3615

Table E5. ADA2 mutations grouped by patient phenotype.

	Vasculitis			PRCA		BMF	
missense	M1T	H112Q	P344L	G47W	G358R	M1T	G321E
	R9W	R169Q	L351Q	H112Q	N370K	G47V	Y353H
	G25C	D238N	A357T	R169Q	M445K	H112Q	G358R
	G47A	L249P	T360A	F178S	L451F	R169Q	Y456C
	G47R	P251L	G450C	L188P	D454H	F178S	V458D
	G47V	W264S	Y453C	F207S	Y456C	L188P	W501R
	I93T	S291L		G321E	Y482C	L311R	
	A109D	E328D					
non-sense		R312X			R306X	Y220X	S265X
						W399X	
Indels		R49Gfs4*			R49Gfs4*	R49Afs*13	F212del
		I143Sfs*41			R49Afs*13	A261Pfs*2	K449NFs*2
Splicing		c.753G>A			c.47+2T>C	c.882-2A>G	
		c.973-2A>G			c.1443-2T>A		
Other		exon7 deletion			exon7 deletion		
		28kb large deletion					
		-144delC promoter deletion					

Table E6. Primer sequences for site directed mutagenesis of ADA2 construct

Mutation	Forward Primer	Reverse Primer
M1T	gaattcaccacgttggtgatg	tgacagatccagcacag
R9W	cccatctgagtgccagccct	ccatccaccaacatgggtaattctg
G25C	gtcttctctgctcagctctatcc	attgccacagccaacagc
G47A	gatgaggctggcggggcggctg	atctttcttcaacaacagatgccccgtg
G47R	ctgagggggcggctggtgctgaa	ccgcatcatctttcttcaacaacagatgcccc
G47V	gatgaggctggtggggcggctg	atctttcttcaacaacagatgccccgtg
G47W	gatgaggctggtggggcggctg	atctttcttcaacaacagatgccccgtg
R49Gs4*	gctggggggggctggtgctg	cgcatcatctttcttcaacaacagatgc
R49A fs*13	cggctggggggcggctggtg	catcatctttcttcaacaacagatgcccc
I93T	aagcatctactgagagaagtc	ggcctgaaaaagtgc
A109D	ctgcacctccatgacattgg	gcatccccctttggcatcatcc
H112Q	ggctgcctgacgctccatgaca	cctttggcatcatccttagaatattaacactg
I143Sfs*41	tcatgcagttcagattgctcac	ccccctgggggaaaca
R169Q	ggaggattatcagaagcgggtgc	agcagaatccacttggaaac
F178S	gtcactgagctgatgacagcttg	gttctgaccccgtctccg
L188P	gaatttactccggtgaccagc	ctcagcaagctgtcatcaaac
F207S	ctggtcgaaatctgaaaccatct	acaacattttggttgtgtaaatc
I210Tfs*57	cttctcaccatctctg	tgglttcaaatttcgacc
F212del	accatctctggtctcatc	gaagatggttcaaatttcg
Y220X	agaagaggatctgtgagcaccagtggtcagagac	gagatgagttttgttcagtgatgagaccagagatg
Y227fs*27	gtcttccggagcatgca	gtctctgaacactgggtgc
D238N	gttctacgagaacaacgtgctctac	tctctgcatgcccgaag
L249P	agagccagcctctgcccgtgt	gatctccatgtagagcacgttg
P251L	caggctgctgctggtgatgagct	gctctgactcctatgtagagcacg
D261P fs*2	agagcaccatccataacgaagagtgg	ccactgagctcatacacc
W264S	tgacgaagagctgctgagtgaaactaccaggaag	tgggtctcctcactgagc
S265X	agaagaggatctgtgagtgaaactaccaggaag	gagatgagttttgtcccactctctgcatggg
S291L	aatcatttattggatcacagatc	ttgattccaataaactcagg
R306X	agaagaggatctgtaattggcattgggctccga	gagatgagttttgttcagtgattctgcatgacagcc
L311R	gcatggggcggcgaatcaagt	cattcgatggattctgcatg
R312X	agaagaggatctgtaattggcattgggctccga	gagatgagttttgttcagtgattctgcatgacagcc
G321E	ggtggtggcagagtttgacctg	gtggggaactgattcgg
E328D	ggtggggcatgacgacactggcc	aggcacaaccctgccacc
P344L	tctgatgatcctcgcaaggatg	gctcctgtagctcatgcaag
L351Q	ggcgttaagcagccttactc	atccttggcggggatcat
Y353H	taagctgcctcacttctccacgcc	acgccatccttggcgggg
A357T	cttctccacaccggagaaacag	taaggcagcttaacgcca
G358R	tctccacgcccagaaacagac	agtaaggcagcttaacgc
T360A	cggcggagaagcagactggca	tggaagaagtaaggcagcttaac
N370K	catagacaggaagattctggatgc	gaagtaccctgccagct
W399X	agaagaggatctgtgaaaaaggacatccccatag	gagatgagttttgttcggagtaagtctgactgc
M445K	tgaccagctaatggtgcca	tcagagctgatcaccatgg
G450C	tgggtccaaatgctgtcctatg	aacatagctgggtcatcag
K449Nfs*2	ttaggctgtcctatgattc	ttggcaccacaacatagctg
L451F	gccaaggctttctatgattc	accaaacatagctgggtc
D454H	ctgtcctatcattctatgaggtc	cctttggcaccacaacatag
Y453C	aggctgtcctgtgatttctatg	ttggcaccacaacatagctg
Y456C	ctatgatttctgtgaggtcttcatgg	gacaagcctttggacca
V458D	gatttctatgaggacttcatggc	ataggacaagcctttggc
M465fsX	agaagaggatctgtgaaaggctgacctgaggacc	gagatgagttttgtcccccaatgccatgaa
K466Tfs*2	ctgaggacctcaaacagc	gtcatcccccaatgcca
Y482C	ctctatcaagtgcagtaccctgtg	ttcatggccagctgttg
W501R	gaagaagagacgggataagttcatag	cagatttccatgaaagatttttc