SUPPLEMENTARY METHODS:

Patients and controls

DNA, serum samples, and clinical data were collected from patients enrolled in observational, longitudinal cohort studies of polyarateritis nodosa (PAN), granulomatosis with polyangiitis GPA, and microscopic polyangiitis MPA conducted by the Vasculitis Clinical Research Consortium (VCRC) at eight academic medical centers in the United States and Canada. All patients were evaluated using standardized data collection forms. Patients with PAN met the 1990 American College of Rheumatology (ACR) Classification Criteria for PAN and were excluded if there was a history of HBV infection. Patients with GPA met the modified 1990 ACR Classification Criteria for the disease, and patients with MPA fulfilled the 2012 revised Chapel Hill disease definitions for this condition [1-3]. Because the initiation of these clinical protocols predated the discovery of DADA2 [4, 5], some phenotypic data now recognized as associated with DADA2, most notably features related to immunodeficiency or bone marrow failure, were not specifically queried, although the investigator could write-in any atypical features believed to be disease-associated. Patients with DADA2 with biallelic *ADA2* mutations, carriers with monoallelic mutations, and healthy controls were recruited at the Clinical Center of the National Institutes of Health.

All study participants provided consent for the use of their specimens and data. The local institutional review boards approved the study Healthy volunteers and individuals with DADA2 were evaluated at the NIH Clinical Center enrolled in protocol 94-HG-0105 (clinicaltrials.gov identifier NCT00001373), which was approved by the NIDDK/NIAMS Institutional Review Board. Written informed consent was obtained from all research participants for samples that were used for the genetic and functional assays, including the measurement of ADA-2 activity in serum. Six patients with DADA2 (with consistent clinical findings and biallelic pathogenic mutations), six monoallelic carriers, and healthy individuals served as controls to assay the ADA-2 activity in serum (Supplementary Table 4). These individuals were age-matched to the patients with PAN with biallelic mutations, to control for the well-documented effect of age on serum ADA-2 levels [4, 6].

Genetic analysis of ADA2 in patients with GPA or MPA

Up to twelve patient DNA samples were pooled and 93 DNA sequencing libraries, compatible with Illumina sequencers, were generated from the pooled DNA using a custom capture kit (New England Biolabs). The 93 libraries were each uniquely barcoded and pooled together for sequencing on a MiSeq DNA Sequencer (Illumina, Inc.). Data were analyzed according to GATK Best Practices recommendations (version 3.8.1) using the MuTect2 algorithm. Candidate *ADA2* variants were validated by genotyping the individual DNA samples using iPLEX assays (Sequenom, San Diego, California) and time of flight mass spectrometry (Agena Bioscience, San Diego). Amplification and extension primers are available upon request.

Variant classification system

We applied the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) criteria, a variant classification framework widely used in diagnostic laboratories, to classify all *ADA2* variants identified in this study [7]. Rare (minor allele frequency < 0.005 in the genome Aggregation Database, https://gnomad.broadinstitute.org), non-synonymous variants (missense and nonsense) and canonical splice-site variants were included in the analysis. Each identified variant was classified into one of five categories (pathogenic [P], likely pathogenic [LP], uncertain significance [VUS], likely benign [LB], benign [B]) using the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) criteria from 2015 [7]. These criteria use available data such as population frequencies, published variant location, computational/in silico analysis, functional data, segregation data, clinical phenotype and others to quantify/determine variant pathogenicity. For a given variant, the evidence available was collected, evaluated, and then combined according to a scoring system (see Richards et al. 2015, table 5). These criteria are considered the standard of care for molecular diagnostics and pathogenic and likely pathogenic variants are considered disease causing [8, 9].

For instance, a very strong (PVS1) criterion can be applied if the identified variant is a loss-of function variant (nonsense, frameshift, canonical splice site, initiation codon, single or multiexon deletion) in a gene where loss-of function is the accepted mechanism of disease. A strong (PS1) can be applied if the observed variant causes the same amino acid change as a previously established pathogenic variant regardless of nucleotide change. The moderate criterion PM1 can be applied if the variant is located in a mutational hot spot and/or in a functional protein domain (e.g., active site of an enzyme). Supportive evidence would be the co-segregation of the variant with disease in multiple affected family members in a gene known to cause the disease (PP1). In contrast, an allele frequency of >5% in population databases or an allele frequency greater than expected for the specific disorder would be considered as an indication for a benign variation (BA1 or BS1).

A detailed explanation of the classification criteria for pathogenic and benign variants can be found in Richards et al. 2015, tables 3 and 4. In brief:

PVS1: Null variant in gene where LOF is a known mechanism of disease

PS1: Same amino acid change as a previously established pathogenic variant

PS2: De novo variant (both maternity and paternity confirmed)

PS3: Well-established *in vitro* or *in vivo* functional studies

PS4: Prevalence of variant in affected individuals is significantly increased compared with prevalence in controls

PM1: Located in mutational hot spot and/or critical and established functional domain

PM2: Absent from controls (or at extremely low frequency if recessive) in population databases

PM3: For recessive disorders, detected in *trans* with a pathogenic variant

PM4: Protein length changes as a result of in-frame deletions/insertions

PM5: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic

PM6: Assumed *de novo* (without confirmation of paternity and maternity)

PP1: Cosegregation with disease in multiple affected family members

PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

PP3: Multiple lines of computational evidence support a deleterious effect

PP4: Patient's phenotype is highly specific for a disease with a single genetic etiology

PP5: Reputable source recently reports variant as pathogenic

BA1: Allele frequency is >5% in population databases

BS1: Allele frequency is greater than expected for disorder

BS2: Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected

BS3: Well-established in vitro or in vivo functional studies show no damaging effect

BS4: Lack of segregation in affected members of a family

BP1: Missense variant in gene for which primarily truncating variants are known to cause disease

BP2: Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in *cis* with a pathogenic variant in any inheritance pattern

BP3: In-frame deletions/insertions in a repetitive region without a known function

BP4: Multiple lines of computational evidence suggest no impact

BP5: Variant found in a case with an alternate molecular basis for disease

BP6: Reputable source recently reports variant as benign

BP7: A synonymous variant for which splicing prediction algorithms predict no impact

For a given variant, the evidence available for this variant is collected, evaluated and then combined according to a scoring system (see Richards et al. 2015, table 5).

For instance, a variant reaches a pathogenic (P) classification if one of the three following sets of criteria applies:

1. 1 Very strong (PVS1) AND

    a) ≥1 Strong (PS1-PS4) OR

    b) ≥2 Moderate (PM1-PM6) OR

    c) 1 Moderate (PM1-PM6) and 1 Supporting (PP1-PP5)

    d) ≥2 Supporting (PP1-PP5)

2. ≥2 Strong (PS1-PS4)

3. 1 Strong (PS1-PS4) AND

    a) ≥3 Moderate (PM1–PM6) OR

    b) 2 Moderate (PM1–PM6) AND ≥2 Supporting (PP1–PP5) OR

    c) 1 Moderate (PM1–PM6) AND ≥4 supporting (PP1–PP5)

In the present study, four distinct algorithms were applied for the supporting category ‘computational (*in silico*) evidence (PP3/BP4)’. PP3 or BP4 criteria were applied if all four algorithms agreed on the prediction (Supplementary Table 5).

Example: ADA2: c.506G>A, p.R169Q:

For example, we evaluated c.506G>A, p.R169Q using the ACMG/AMP variant classification criteria and applicable criteria for this variant included:

1. PS3 can be applied because there are well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product [5, 10, 11].

2. PM2 can be applied because p.R169Q is absent from controls (or at extremely low frequency if recessive) in population databases (gnomAD: 4.74^{-04} , 1000 Genomes Project 2.0^{-04}) [12, 13].

3. PM3 can be applied because p.R169Q was detected in *trans* with a pathogenic variant in affected individuals [4, 14].

4. PP1 applies because p.R169Q co-segregates with disease in multiple affected family members in a gene definitively known to cause the disease [5, 10].

5. PP3 applies because multiple lines of computational evidence support a deleterious effect on the gene or gene product (SIFT: Damaging, Mutationtaster: Disease causing, Provean: Deleterious, Polyphen-2: Probably damaging) [15-18].

The available evidence can be combined according to the ACMG/AMP 2015 scoring system as follows: 1 Strong (PS3) AND 2 Moderate (PM2, PM3) AND ≥2 Supporting (PP1, PP3) → Pathogenic variant

Measurement of ADA-2 enzyme activity

Serum ADA-2 activity relative to a control group was determined by a spectrophotometric assay using a commercially available kit (Diazyme Laboratories, Poway, CA, USA) by adding ADA1-inhibitor EHNA (erythro-9-Amino-b-hexyl-a-methyl9H-purine-9-ethanol hydrochloride) (100 μM; Sigma-Aldrich, Zwijndrecht, Netherlands) according to the manufacturer's protocol. An ADA calibrator (50.3U/L, Diazyme) was used to generate a linear slope of absorbance vs. ADA2 activity (mU/mL). Serum samples to assay for reduced ADA-2 enzyme activity were available for 88 patients with idiopathic PAN, for 16 patients with GPA or MPA who had monoallelic ADA2 variants, and for 35 randomly chosen patients with GPA/MPA without *ADA2* variants.

For a selected subset of *ADA2*-genotyped patients with idiopathic PAN (n = 11), along with various controls, ADA-2 enzymatic activity was measured by a CLIA-certified laboratory of one of the co-authors (MSH) on stored sera. The complete 50 μL ADA-2 reactions, run in duplicate, contained 5 μL of patient sample (serum or plasma), 100 mM Tris-acetate, pH 7.0; 10 mM adenosine; and 100 μM EHNA. Two single control reactions were also run, each containing 5 μL of sample and buffer, but one lacked EHNA and the other lacked substrate. To perform these assays, patient sample, buffer, and EHNA or water (no EHNA control) were combined and equilibrated at 37°C for 5 minutes; then the reactions were initiated by adding adenosine to 3 tubes and water to the no substrate control tube. After further incubation for 120 min, reactions were terminated by adding 12.5 μL of cold 5N perchloric acid, followed by centrifugation (4C at 20,000 x g for 7min). Forty (40) μL of each supernatant was then neutralized with 12.5 μL of 3N KOH, 1M $KHCO_3$. After centrifugation as above, 30 μL of the neutralized supernatants were analyzed on a Waters C_{18} μBondapak (3.9 x 300 mm) HPLC column equilibrated and eluted with 0.05 M ($NH_4H_2PO_4$, 8% methanol, 1% acetonitrile, pH 5.2. As previously described [4], both inosine and hypoxanthine were quantified (the latter is formed from inosine by purine nucleoside phosphorylase present in patient samples in variable amounts). ADA2 activity (i.e. the sum of inosine plus hypoxanthine formed from adenosine during incubation in the presence of EHNA) was expressed as nmols per min (milliunits) per mL of undiluted plasma. Reference ranges were determined for patients with DADA2 , carriers, and healthy controls who had been identified based on clinical data and, in some cases, DNA sequencing.

Reference values [mean mU/mL ± sd (range)] are as follows: DADA2 patients (n=55), 0.4 ± 0.5 (0 – 2.5); ADA2 carriers (n = 46), 5.7 ± 1.9 (2.9 – 11.4); healthy controls (n = 27 + pooled human plasma), 13.0 ± 5.1 (4.7 – 27.2).

In the CLIA certified laboratory involved in the present investigation, a validated assay of plasma ADA-2 enzymatic activity has accurately diagnosed DADA2 in the 80% of cases where results of *ADA2* gene sequencing have been provided. The range of plasma ADA-2 activity has been established for authentic carriers, i.e. parents of patients with DADA2 and sibs shown to be carriers by gene sequence analysis. However, as there is overlap with the lowest plasma ADA-2 activity found in healthy controls, when enzyme activity is found to be within the carrier range, *ADA2* gene sequence analysis is advised as necessary to establish heterozygote status. In true

heterozygous carriers, certain factors, such as infection, may induce increased production of the ADA-2 protein from the wild type allele [19]. The situation may be further obscured if, as some postulate, there may be biologic functions of the ADA-2 protein that are not measured by the enzymatic assay, which may affect the clinical phenotype. These considerations all underscore the view that sequencing and functional assays are complementary, and that both may be required for a complete clinical evaluation.

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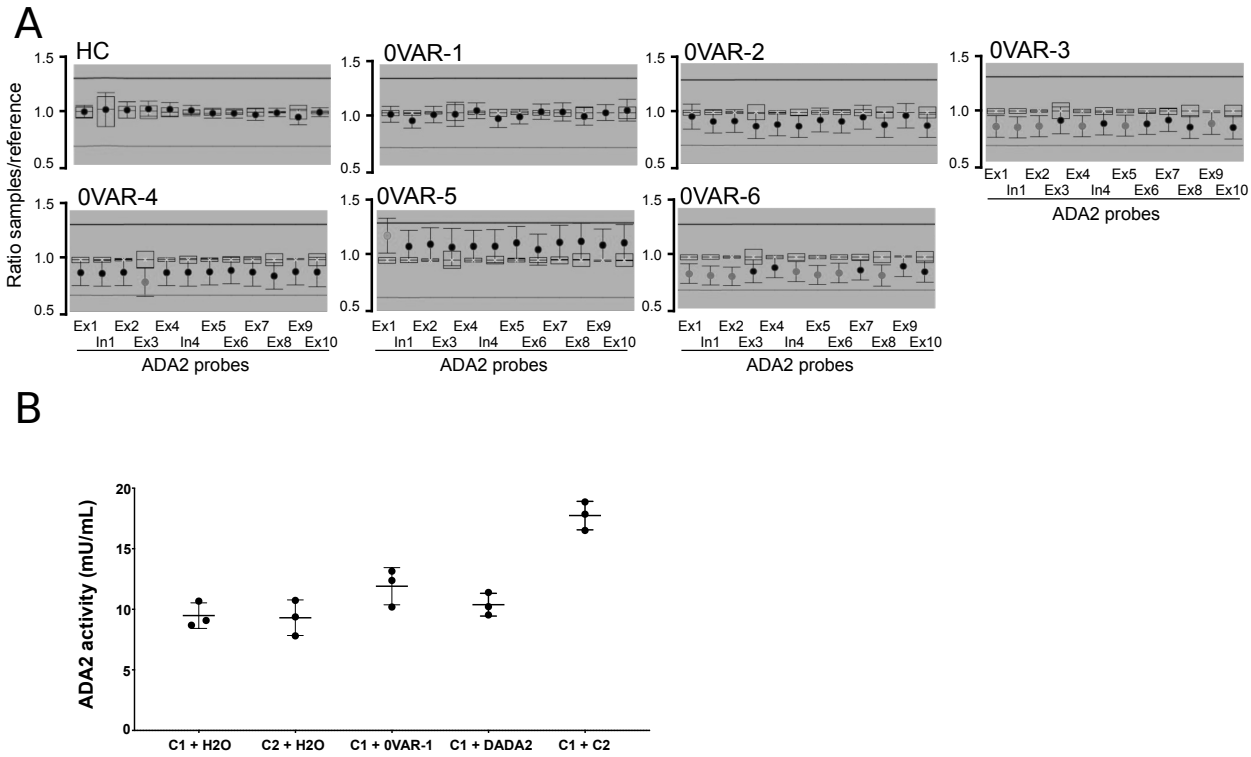
SUPPLEMENTARY FIGURE:

Supplementary Figure 1:



A: Multiplex ligation-dependent probe amplification (MLPA) analysis of the *ADA2* locus in healthy control and six patients with PAN (0VAR-1 – 0VAR-6) with reduced ADA2 enzyme activity but without identifiable pathogenic variants by Sanger sequencing. *ADA2* probes are depicted on the X axis and on the Y axis, the ratio of relative fluorescence units (RFU) of the specific *ADA2* probe and the RFU value of the reference probes are shown. A ratio above the top line (dark) or below the bottom line (light) would be interpreted as evidence for copy number variation. Ex: exon, In: intron, HC: Healthy control.

B: Serum mixing assays to evaluate the potential presence of an inhibitor of ADA-2. Sera from 2 healthy controls (C1, C2), patient 0VAR-1, and a patient with confirmed DADA2 (G383S/G383S) were used. Five µl of control serum (C1) was either mixed with 5 µl ddH2O or the same amount of appropriate patient serum. ADA-2 activity was determined by a commercial spectrophotometric assay (Diazyme). The fact that the ADA-2 activity measured for C1 + 0VAR-1 is not significantly less than C1 +H_2O argues against the presence of a soluble ADA-2 inhibitor in the serum of 0VAR-1.

SUPPLEMENTARY TABLES:

Supplementary Table 1: Demographic characteristics of the subjects enrolled in the observational cohort studies of PAN and GPA or MPA

Characteristics	PAN cohort (N=118)	GPA/MPA cohort (N=1107)
Age (years) - Mean (range)		
At onset	42.7 (9.1-74.7)	46.5 (5.0-93.5)
At diagnosis	44.6 (10.6 - 75.3)	57.6 (8.5-93.5)
Sex (%)		
Female	58 (49.2)	592 (53.5)
Race (%)		
White	102 (86.4)	1016 (91.8)
Asian	7 (5.9)	33 (2.9)
Black or African American	3 (2.5)	21 (1.9)
More than one race	3 (2.5)	10 (0.9)
American Indian/Alaska Native	2 (1.7)	5 (0.5)
Unknown or Not Reported	1 (0.8)	21 (1.9)
Native Hawaiian or Pacific Islander	0 (0.0)	1 (0.1)
Ethnicity (%)		
Not Hispanic, Latino, or Spanish origin	105 (89.0)	1002 (90.5)
Hispanic, Latino, or Spanish origin	5 (4.2)	40 (3.6)
Unknown or not reported	8 (6.8)	64 (5.8)

PAN: polyarteritis nodosa; GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis

Supplementary Table 2: Subjects with GPA/MPA and rare monoallelic variants in *ADA2*

Patient	Sex	Age at onset	Age at diagnosis	Age at study entry	Nucleotide change	Amino acid substitution	MAF	ACMG classification	Serum available
GM1	F	33.2	34.8	34.9	c.63G>A	p.M21I	4.07E-06	VUS (PM1, PM2, BP4)	no
GM2	M	54.3	54.3	57.1	c.134G>A	p.R45Q	8.30E-05	VUS (PM1, PM2, BS2, BP4)	yes
GM3	M	60.2	60.4	61.1	c.194C>T	p.T65M	6.31E-04	VUS (PM1, PM2)	no
GM4	F	59.0	60.2	61.1	c.194C>T	p.T65M	6.31E-04	VUS (PM1, PM2)	no
GM5	F	55.8	58.8	61.5	c.250A>G	p.M84V	1.41E-04	VUS (PM2, BP4)	yes
GM6	F	65.7	66.0	66.2	c.316C>T	p.P106S	1.63E-05	LP (PM1, PM2, PP3, PP5)	yes
GM7	M	20.4	20.4	31.8	c.362T>C	p.M121T	1.34E-04	VUS (PM2, BS2, BP4)	no
GM8	M	60.8	.	62.6	c.435G>C	p.Q145H	4.33E-05	VUS (PM1, PM2)	yes
GM9	M	32.1	32.3	34.5	c.511C>T	p.R171W	1.54E-03	VUS (PM1, BS2, BP6)	yes
GM10	M	74.0	76.3	77.1	c.511C>T	p.R171W	1.54E-03	VUS (PM1, BS2, BP6)	yes
GM11	F	37.4	38.2	41.1	c.832G>A	p.E278K	3.23E-05	VUS (PM2, BP4)	no
GM12	M	57.7	57.7	67.9	c.927G>A	p.M309I	1.67E-03	LB (BS2, BP6)	yes
GM13	M	69.6	69.6	74.7	c.927G>A	p.M309I	1.67E-03	LB (BS2, BP6)	no
GM14	F	30.8	32.1	40.4	c.927G>A	p.M309I	1.67E-03	LB (BS2, BP6)	no
GM15	F	70.8	70.9	75.8	c.973-2A>G	NA	1.27E-04	LP (PVS1, PM2)	no
GM16	F	41.4	42.0	43.1	c.1045G>A	p.V349I	2.14E-03	VUS (PM1, PM2, BP4, BP6)	yes
GM17	M	65.2	65.7	68.0	c.1045G>A	p.V349I	2.14E-03	VUS (PM1, PM2, BP4, BP6)	yes
GM18	M	25.9	25.9	32.9	c.1045G>A	p.V349I	2.14E-03	VUS (PM1, PM2, BP4, BP6)	no
GM19	M	54.0	54.3	57.1	c.1045G>A	p.V349I	2.14E-03	VUS (PM1, PM2, BP4, BP6)	yes
GM20	F	42.4	42.6	46.0	c.1045G>A	p.V349I	2.14E-03	VUS (PM1, PM2, BP4, BP6)	no
GM21	F	70.6	71.0	80.6	c.1045G>A	p.V349I	2.14E-03	VUS (PM1, PM2, BP4, BP6)	yes
GM22	M	54.4	54.6	57.2	c.1045G>A	p.V349I	2.14E-03	VUS (PM1, PM2, BP4, BP6)	no
GM23	M	73.7	73.9	74.1	c.1110C>A	p.N370K	NA	VUS (PM2, PP3, PP5)	yes
GM24	M	61.6	61.7	61.8	c.1442+2T>G	NA	NA	LP (PVS1, PM2)	yes

Patient	Sex	Age at onset	Age at diagnosis	Age at study entry	Nucleotide change	Amino acid substitution	MAF	ACMG classification	Serum available
GM25	F	17.4	17.6	28.4	c.1465G>C	p.E489Q	1.30E-04	VUS (PM2, BP4)	yes
GM26	M	45.9	46.4	49.3	c.1467G>C	p.E489D	3.79E-04	VUS (PM2, BP4)	yes
GM27	F	39.6	45.8	46.1	c.1467G>C	p.E489D	3.79E-04	VUS (PM2, BP4)	yes

For each of the identified variants the change in coding sequence (nucleotide change), protein change (amino acid substitution), minor allele frequency (MAF) as reported by genome Aggregation Database (gnomAD), ACMG/AMP classification result (ACMG classification), and serum availability is shown. P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance; PM, pathogenic moderate; PP, pathogenic supporting; BS, benign strong; BP, benign supporting; N.A., Not available; M, male; F, female; See Supplementary Methods for variant classification and for definition of PVS1, PM1, PM2, PP3, PP5, BS1, BS2, BP4, and BP6.

GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis;
MAF: minor allele frequency; ACMG: American College of Medical Genetics and Genomics

Supplementary Table 3: Clinical features among GPA/MPA subjects with and without detectable pathogenic variants in *ADA2*

	Monoallelic *ADA2* Mutation n = 27	No *ADA2* Mutation n = 1080	P value
Age at symptom onset years, mean, SD	50.4 (16.6)	46.1 (17.7)	0.20
Age at diagnosis years, mean, SD	50.8 (16.8)	47.2 (17.4)	0.29
Female sex	12 (43%)	580 (54%)	0.34
White	23 (82%)	993 (92%)	0.07
Cardiac	1 (4%)	50 (5%)	1.00
Constitutional	24 (86%)	871 (81%)	0.63
Cutaneous	9 (32%)	340 (32%)	1.00
Ear, Nose, Throat	20 (71%)	862 (80%)	0.34
Gastrointestinal	0 (0%)	38 (4%)	0.62
Kidney	19 (68%)	677 (63%)	0.69
Lung	20 (71%)	747 (69%)	1.00
Musculoskeletal	20 (63%)	720 (67%)	0.69
Nervous System	5 (16%)	238 (22%)	0.82
Ocular	9 (32%)	328 (30%)	0.84

SD, Standard deviation. There were no significant differences in cutaneous features between patients with monoallelic *ADA2* mutations compared to those without *ADA2* mutations in terms of gangrene (3 vs 0%); livedo reticularis (3 vs 0%); nodules (3 vs 0.2%); purpura (3 vs 4%); Raynaud's phenomenon (3 vs 0%); or ulcer (3 vs 2%).

GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis

Supplementary Table 4: ADA2 enzymatic activity in individuals serving as controls for this study, measured by HPLC and spectrophotometry

#	Control	Sex	Age at serum collection	Amino acid substitution(s)	ADA2 activity (HPLC) [mU/mL]	ADA2 activity (Spec.) [mU/mL]
1	DADA2 1	F	23	p.E328K/p.F355L	1.5	0.9
2	DADA2 2	F	28	p.R34W/p.H112Q	0.8	0.0
3	DADA2 3	F	17	p.G47A/p.H112Q	2.5	1.3
4	DADA2 4	F	11	p.F178S/p.F178S	0.8	0.5
5	DADA2 5	F	24	p.Y453C/c.-47+2T>C	0.2	0.0
6	DADA2 6	M	19	p.G47A/p.G47A	1.9	1.9
7	Carrier 1	M	20	p.G47A/WT	13.5	9.2
8	Carrier 2	M	18	p.R169Q/WT	6.9	5.5
9	Carrier 3	M	13	p.R306X/WT	9.4	7.6
10	Carrier 4	M	14	p.R169Q/WT	8.2	6.7
11	Carrier 5	F	16	p.G47A/WT	5.4	4.9
12	Carrier 6	F	17	p.Y453C/WT	11.5	8.5
13	Control 1	F	12	WT/WT	23.8	14.8
14	Control 2	M	23	WT/WT	12.7	8.7
15	Control 3	F	20	WT/WT	12.6	10.0
16	Control 4	F	14	WT/WT	16.0	11.8
17	Control 5	F	16	WT/WT	13.2	8.0
18	Control 6	M	30	WT/WT	8.5	8.4

ADA-2 enzymatic activity was measured in mU/mL by an HPLC-based method (HPLC) and by using the Diazyme commercial spectrophotometric assay (Spec.) on stored sera. DADA2, Patients with biallelic DADA2, evaluated at the NIH Clinical Center; Carrier, Individuals carrying one pathogenic variant in *ADA2,* evaluated at the NIH Clinical Center*;* Control, Healthy control individuals, evaluated at the NIH Clinical Center. HPLC assay reference ranges [mean mU/mL ± sd (range)] are as follows: Patients with DADA2 (n=55), 0.4 ± 0.5 (0 – 2.5); ADA2 carriers (n = 46), 5.7 ± 1.9 (2.9 – 11.4); healthy controls (n = 27 + pooled human plasma), 13.0 ± 5.1 (4.7 – 27.2).

Supplementary Table 5: *In silico* prediction of identified variants on ADA-2 protein function

Subject	Nucleotide change	Amino acid substitution	MAF	Sift	Mutation-taster	Provean	Polyphen
GM1	c.63G>A	p.M21I	4.07E-06	T	T	T	T
1VAR-1	c.100C>T	p.R34W	7.78E-05	D	T	D	D
GM2	c.134G>A	p.R45Q	8.30E-05	T	T	T	T
2VAR-4	c.139G>T	p.G47W	3.98E-06	D	D	D	D
2VAR1	c.140G>C	p.G47A	6.01E-05	T	D	D	D
1VAR-2/GM3/GM4	c.194C>T	p.T65M	6.31E-04	T	T	T	T
GM5	c.250A>G	p.M84V	1.41E-04	T	T	T	T
2VAR-1/GM6	c.316C>T	p.P106S	1.99E-05	D	D	D	D
GM7	c.362T>C	p.M121T	1.34E-04	T	T	T	T
GM8	c.435G>C	p.Q145H	4.33E-05	T	T	T	D
2VAR-4	c.506G>A	p.R169Q	4.74E-04	D	D	D	D
GM9/GM10	c.511C>T	p.R171W	1.54E-03	D	T	D	D
GM11	c.832G>A	p.E278K	3.23E-05	T	T	T	T
1VAR-3/GM12-GM14	c.927G>A	p.M309I	1.67E-03	T	T	T	T
GM15	c.973-2A>G	N.A.	1.27E-04	N.A.*	N.A.*	N.A.*	N.A.*
2VAR-2	c.982G>A	p.E328K	N.A.	D	D	D	D
1VAR-4/GM16-GM22	c.1045G>A	p.V349I	2.14E-03	T	T	T	T
2VAR-2	c.1065C>A	p.F355L	2.34E-04	T	D	D	D
GM23	c.1110C>A	p.N370K	NA	D	D	D	D
2VAR-3	c.1147G>A	p.G383S	3.58E-05	D	D	D	D
1VAR-5	c.1358A>G	p.Y453C	8.84E-05	D	D	D	D
GM24	c.1442+2T>G	N.A.	N.A.	N.A.*	N.A.*	N.A.*	N.A.*
GM25	c.1465G>C	p.E489Q	1.30E-04	T	T	T	T
GM26/GM27	c.1467G>C	p.E489D	3.79E-04	T	T	T	T

Subjects with the 2VAR, 1VAR, and 0VAR designations are from the PAN cohort. Subjects with the GM designation are from the GPA/MPA cohort. *In silico* modeling using four distinct tools (SIFT, Mutationtaster, Provean, Polyphen) was used to predict the possible impact (Damaging (D) or Tolerated (T)) of *ADA2* gene variants identified in individuals with PAN, GPA, and MPA [15-17, 20]. For each subject, the change in the coding sequence (nucleotide change), the protein change (amino acid substitution), and the minor allele frequency (MAF) as reported by genome Aggregation Database (gnomAD) is shown. N.A., not available; *, applied algorithms are not applicable for splicing variants.