SUPPLEMENTARY METHODS

Genetic analysis

Whole-exome sequencing (WES) was performed on 50ng of genomic DNA extracted from peripheral blood of 4 cases (A-5, B-1 & 2 and C-1). Library preparation was completed using the Illumina Nextera Rapid Capture Exome Library Preparation Kit. Sequencing was completed using the Illumina HiSeq 1000 platform. Sequence data were mapped using Galaxy (1-3) and Human Genome Reference Consortium build 37/ hg19 (GRCh37/hg19) as a reference. Over 95% of reads were aligned to the reference genome. SNPs were called using wANNOVAR (4).

Homozygosity mapping was performed in the index case of family A (A-5), the parents, the symptomatic sibling and the asymptomatic siblings by using the Illumina CytoSNP12 SNP array and analysed using Illumina Genome Studio 2011.1 software with loss of heterozygosity detector plug in to identify runs of homozygosity (version 1.9.0).

Sanger sequencing for *CECR1* was performed using the primers shown in table S-1. FastStart PCR Master mix (Roche) was used for amplification and BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystem) was used for the sequencing reaction. Sanger sequencing was performed using the Applied Biosystems 3730 DNA Analyzer and calls were made using Applied Biosystem 5.2 software. The sequencing output was analysed using CodonCode Aligner 5.1 to align and visualize the reads.

Table S-1 Sequences of forward and reverse primers used for Sanger sequencing of all the exons and untranslated regions of *CECR1*

	Forward primer	Reverse Primer
1	TTGGTGTGAAGAAAGGGATG	TGTGTGATGTTCCCCTTGAA
2	CAGGGCGAGAAAAGGAAGAT	TGAGGCTTTCTCTGCTCCAT
3	GACTTCACCCCCTCCTTTGT	GCTGGGATTATAGGCGTGAA
4	AAGTTCGTCGCTTTCCTCAC	TATGCACTCCCACACCAAGA
5	TTTGGGTTTAAGCAGGCAGT	TCCATTTCAGGTTTCCTTGC
6	CCCCTGACCCCATCTACTTT	GGACTGTGCTCTCACACAGG
7	GCCAGCAAGGGTCTTTGATA	GGGCTGGTGAGGGTTTTAAG
8	GGCTGTAGTGCATTGGTGTG	ACAGGAAAGGGCTCTGGAAA
9	GGAAACTGTGTGTGTGTATTTGTG	GCAAAAAGTTTAAGAGAGCAGAGG
10	GGAGCTGATGGGGCTCAA	ACCCACAGGAACCATCGAG
11	TGGCTGTTTAGTCCTTGCTG	TGTGAGCTCTCCAAGTGCAT
12	ATGCCTGGCCTATTCTGGTC	GGACCACGAGGTCAGGTG
13	GGTTCAAGCGGATTCTCGT	TTCTTTGATTCCCTGCCATC

Adenosine deaminase 2 assay

Diazyme's ADA Assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. One unit of ADA was defined as the amount of ADA that generates one μmole of inosine from adenosine per minute at 37°C. This kit cannot differentiate the function of ADA type 1 or 2. In order to

specifically measure ADA2 activity, 100 nM of specific ADA1 inhibitor; erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), was added during the assay. Reading of the absorbance of the enzyme activity using the Fluorstar Optima spectrophometer at wavelength 540 nm with lens. Intra-test coefficient of variation (CV) is < 4.5% and inter-test CV is < 5% (manufacturer's product sheet).

SUPPLEMENTARY RESULTS

Figure S-1 Family tree of family B, C and G. All genotypes identified by Sanger sequencing. WT: wild type

A: Family B. Patient B-1 and B-2 have homozygous p.Gly47Arg (p.G47R/ p.G47R) mutations and the parents are heterozygous for this variant (p.G47R/WT).

B: Family C. Patient C-1 and C-2 have compound heterozygous p.Pro251Leu/c.-114DelC (P251L/c.-114DelC) mutations. The mother and healthy sister are heterozygous (P251L/WT), and the father is heterozygous (c.-114DelC/WT).

C: Family G. Patient G-1 and G-2 were homozygous for the mutation p.Arg169Gln (p.R169Q/ p.R169Q), no samples from the parents were available for sequencing.

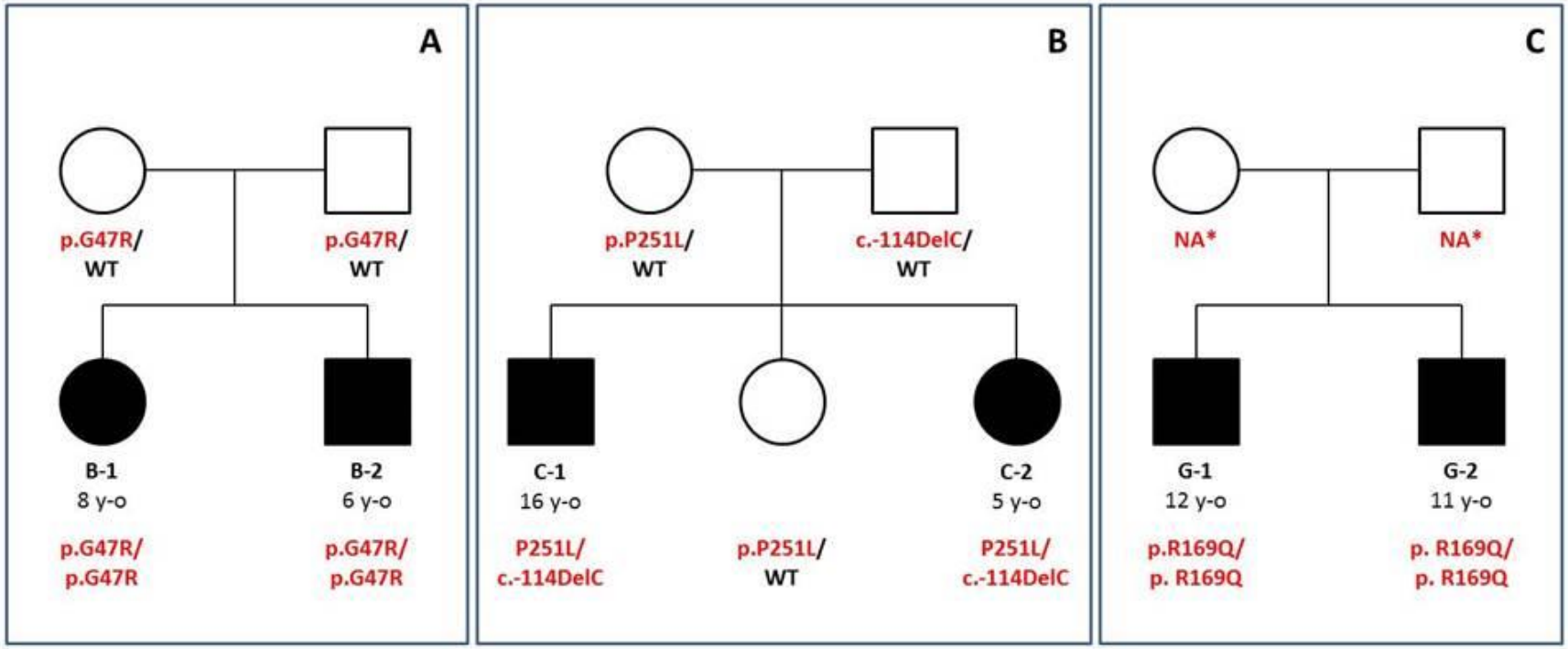
A
p.G47R/
WT
p.G47R/
WT
B-1
8 y-o
p.G47R/
p.G47R
B-2
6 y-o
p.G47R/
p.G47R
B
p.P251L/
WT
c.-114DelC/
WT
C-1
16 y-o
P251L/
c.-114DelC
p.P251L/
WT
C-2
5 y-o
P251L/
c.-114DelC
C
NA*
NA*
G-1
12 y-o
p.R169Q/
p. R169Q
G-2
11 y-o
p. R169Q/
p. R169Q

Table S-2: Histopathological and angiographic findings in 9 symptomatic DADA2 patients

Patient	Histopathological features	Angiographic findings
A-5	Sural nerve biopsy|: Epineurial vessels were infiltrated by inflammatory cells with associated disruption of the elastic lamina, patchy active and mild axonal neuropathy with minimal regeneration	MRA whole aorta: Normal
B-1	Skin biopsy: reactive endothelial changes with distortion of the capillary luminae, segmented leucocyte and lymphocytic cuffing and associated mild perivascular oedema.	NA
B-2	Skin biopsy: infiltration of neutrophils and eosinophils with leucocytoclasis in dermal blood vessels with destruction of vessels	MRI/MRA brain and brain stem normal
C	NA	MRI of the leg with contrast: multiple tortuous blood vessels with phlebolith within
D	NA	DSA: truncation and beading with multiple microaneurysms in intrarenal arteries, jejunal branches of superior mesenteric artery and intrahepatic arteries.
E	Skin biopsy: thrombosis, with fibrin deposition within the wall and concentric active inflammation with plasma cells, lymphocytes and neutrophils in subcutis blood vessels	DSA: multiple microaneurysms in both kidneys and in the liver
F	NA	MRA: multiple aneurysmal dilatation of the small intra-renal vessels DSA: multiple microaneurysms in both kidneys
G-1	Skin biopsy: normal	MRA brain (6 years old): Cerebral haemorrhage at right frontal area with multiple pseudoaneurysms in left basofrontal area. MRA brain (6 1/2 years old):multiple infarctions in pons
G-2	Skin biopsy: normal	MRA brain: Acute hemorrhagic stroke left basal ganglia with hemiplegia and transient aphasia, no evidence of aneurysms

DSA: digital subtraction angiography, MRA: magnetic resonance angiogram, NA: not applicable

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