Online Supplemental Material

Online Materials and Methods

Neuroimaging studies and protocols

All patients presented at least one brain MRI for review (median 4 studies, range 1-12). Median age at first MRI was 7.8 years (range 1.4-20.8 years). Longitudinal studies were available in 10/12 cases (median imaging follow-up time of 5.5 years; range: 11 months-13.9 years). Patients were scanned on 1.5T or 3T MR units with different imaging protocols, all including DWI, T1WI, T2WI and FLAIR sequences and underwent at least one angiographic study, i.e. CTA, MRA and/or DSA (median 3, range 1-10). T2*-weighted sequences were available in 8/12 patients. Contrast material was injected in 10/12 subjects, including twenty-four studies with additional post-contrast fat sat T1WI and/or black-blood vessel-wall imaging sequences. Finally, arterial spin labelling studies were performed in 7/12 patients (range:1-5). In total, 59 brain MRI, 3 spinal MRI, 48 MRA, 9 head CT scans, 2 CTA and 4 DSA studies were analyzed.

Online Tables

Online Table 1. Clinical and genetic features and treatment response

	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Sex	M	M	M	F	F	F	F	M	M	F	M	F
Current age	20Y	12Y	13Y	11Y	18Y	40Y	22Y	17Y	25Y	31Y	24Y	25Y
Age at symptoms onset	2Y	9mo	6mo	3mo	5Y	5Y	1Y	1Y	7Y	3mo	3Y	20mo
Age at genetic diagnosis	15Y	7Y	9Y	5Y	17Y	35Y	17Y	13Y	19Y	16Y	14Y	20Y
ADA2 mutation	R312X/ E328D	R312X E328D	T360A T360A	22q11.1 duplication	G47R G47R	T360A T360A	T360A T360A	L249P P344L	L249P T360A	T360A/ T360A	G47V S479P	G47V S479P
Origin	Italian	Italian	Italian	Italian	Asian	Italian	Italian	Italian	Italian	Italian	Italian	Italian
Family history	Yes (brother: patient #2)	Yes (brother: patient #1)	No	No	Unknown (adopted)	Yes (sister: patient #7)	Yes (sister: patient #6)	Yes (brother)	No	Yes (uncle & brother)	Yes (sister: patient #12)	Yes (brother: patient #11)
Endogamy*	Yes	Yes	Yes	No	Unknown (adopted)	No	No	No	No	No	Yes	No
Deficiency of serum ADA2 activity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	Yes	Yes
Clinical presentation	Livedo reticularis	Fever, livedo reticularis	L facial paralysis	Livedo reticularis	Skin vasculitis	Fever, arthralgia	Skin rash	Fever, arthralgia, abdominal pain	Fever, seizures	Fever	Vertigo	Ataxia

Clinical course	Chronic	Chronic	Chronic	Chronic	Chronic	Chronic	Chronic	Chronic	Recurrent	Chronic	Chronic	Chronic
Biopsy findings	PAN (skin)	PAN (bowel)	NA	NA	NA	PAN (skin)	PAN (skin)	NA	Necrotizing vasculitis (bowel)	Leucocytoclastic vasculitis/ PAN (skin)	NA	Leucocytoclastic vasculitis/ PAN (skin/bowel)
Fever	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No
Elevation of acute phase reactants	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Immunodeficiency	Hypogammaglobulinemia	Hypogammaglobulinemia	No	Hypogammaglobulinemia	No	No	No	No	No	No	Hypogammaglobulinemia	Hypogammaglobulinemia, recurrent upper airway infection
Arterial hypertension	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Dermatological findings	Livedo reticularis, subcutaneous nodules	Livedo reticularis	Livedo reticularis	Livedo reticularis	Livedo reticularis, skin ulcers	Livedo reticularis, subcutaneous nodules, skin ulcers	Livedo reticularis, skin ulcers	Livedo reticularis	Livedo reticularis, skin ulcers	Livedo reticularis	Livedo reticularis, subcutaneous nodules, skin ulcers	Livedo reticularis, subcutaneous nodules
Presenting neurological symptoms/signs (age)	Diplopia, anisocoria, ptosis, strabismus (6Y)	R hemiplegia (17mo)	L facial paralysis, diplopia (6mo)	R Hemiparesis, R blindness (4Y)	L hemiparesis, coma (10Y)	L hemiparesis (12Y)	Focal sensitive-motor abnormalities (2Y)	Seizures (8Y)	Seizures (7Y)	Diplopia, palpebral ptosis, strabismus (7Y)	Vertigo (3Y)	Ataxia (20mo)

Age at first documented stroke	6Y	17mo	6Y	4Y	10Y	12Y	2Y	NA	NA	7Y	9Y	12Y
CNS recurrences	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	Yes
Sensorineural Hearing Loss	No	No	Yes	No	No	No	No	Yes	No	No	No	No
Cranial nerve involvement	III nerve palsy	No	III + VII nerve palsy	Optic nerve neuritis	No	No	No	No	No	III nerve palsy	No	No
Other relevant clinical findings	Arthralgia	Diarrhea, bowel perforation	Myocarditis	Myocarditis	Arthralgia	Arthralgia, Hepatosplenomegaly	Arthralgia	Episcleritis Diarrhea, abdominal pain, arthralgia	Bowel invagination, arthralgia	No	Colitis	Colitis
Current immunosuppressive treatment (age at introduction)	Etanercept (9Y)	Etanercept (4Y)	Etanercept (7Y)	Etanercept (4.5Y)	Etanercept (16.5Y)	Thalidomide (13Y)	Thalidomide (13Y)	Etanercept (13Y)	Etanercept (14Y)	Thalidomide (14Y)	Etanercept (18Y)	Etanercept (20Y)
Clinical response to current treatment	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete
Previous immunosuppressive treatment(s)	Thalidomide + CPP + steroids	Thalidomide+ steroids	Steroids	Anakinra + steroids	Steroids	MTX + AZA + MPL + steroids	CPP + AZA + MPL + steroids	Steroids	CPP + MPL + steroids	MTX + infliximab + steroids	AZA + MPL + steroids	CPP + MPL + steroids
Age at last clinical follow-up	20Y	13Y	13Y	12Y	19Y	38Y	21Y	17Y	22Y	17Y	24Y	25Y

Legend: AZA- Azathioprine, CPP- Cyclophosphamide, F- Female, L- Left, M- Male, mo- months, MPL- Mycophenolate, MTX- Methotrexate, NA- Non applicable, PAN- Polyarteritis nodosa, Y- Years

***** patients without a clear consanguinity but whose parents have their ancestor in the same small village

Online Table 2. Neurological and neuroimaging characteristics at first available MRI and at follow-up

Pt	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**#11**	**#12**
Age at first available brain MRI	6Y + 1m	17mo	6Y + 2mo	5Y + 10mo	16Y + 6mo	20Y + 2mo	3Y + 3mo	7Y + 7mo	7Y + 7mo	19Y + 1mo	18Y + 4mo	20Y + 2mo
Ischemic stroke	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes
- **Multiple lesions**	Yes	No	Yes	Yes		Yes	Yes			No	Yes	Yes
- **Circulation(s) involved**	A & P	A	P	A & P		A	A & P			P	A	A & P
- **Location and side**	L cerebral peduncle (2), L nucleo-capsular region (2)	L nucleo-capsular region	R midbrain (3), R pons	L thalamus, R nucleo-capsular region		Bilateral nucleo-capsular region (4), multiple periventricular and deep WM lesions, isthmus CC	L cerebral peduncle (2), L centrum semi-oval			R cerebral peduncle (1)	L nucleo-capsular region, R fronto-parieto-occipital region	Bilateral thalamus (5), R midbrain, bilateral nucleo-capsular region (3)
- **At least one acute (DWI+) lesion**	Yes	No	No	Yes		NA	NA			NA	No	No
Hemorrhagic stroke	Yes- L temporal lobar hemorrhage	No	No	No	Yes - R fronto-parietal lobar hemorrhage, SAH	No	No	No	No	No	No	No

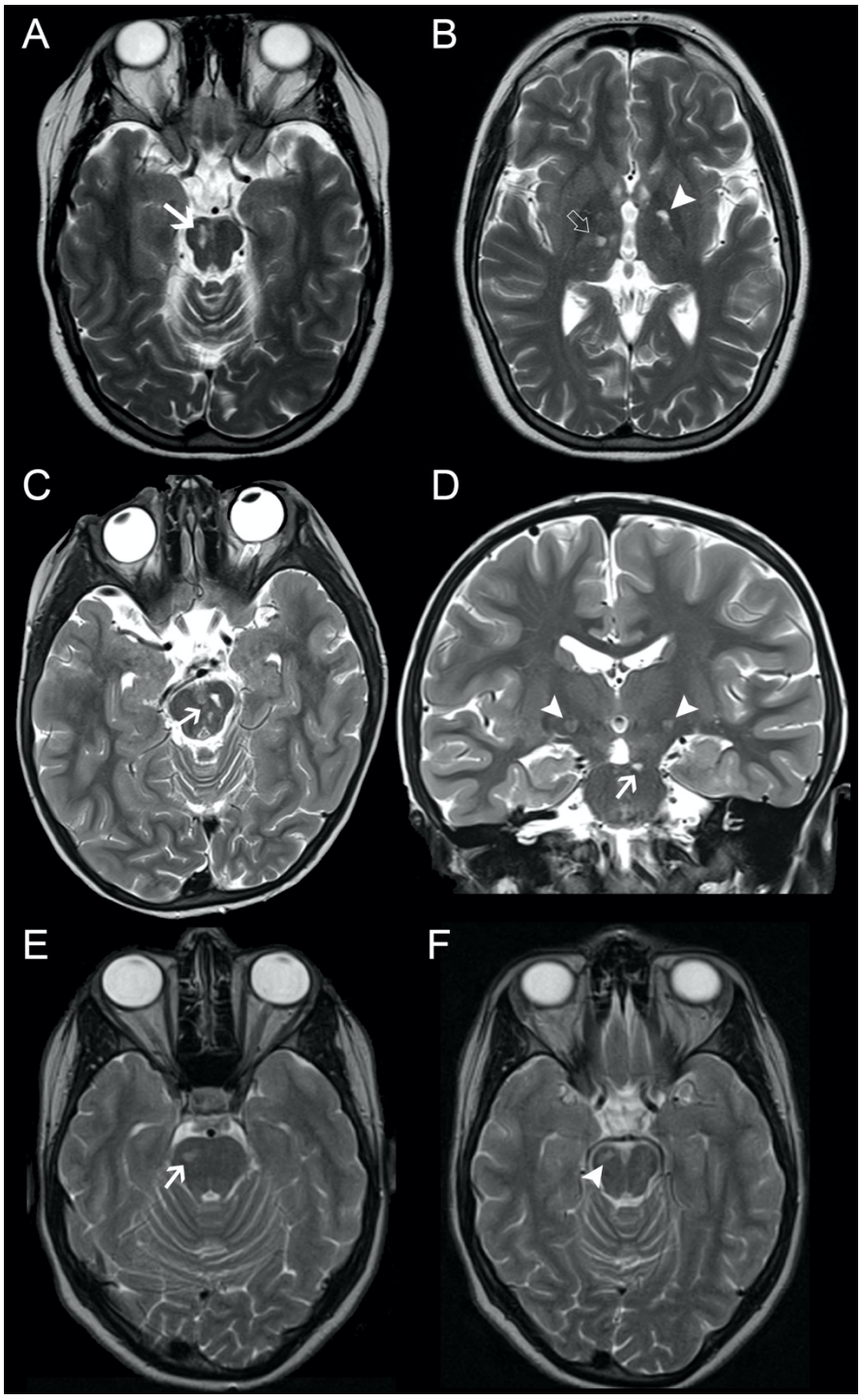
Focal areas of susceptibility effect on T2*WI/SWI	Yes- R parietal region	No	No	Yes- R putamen	Yes- superficial siderosis	NA	NA	NA	NA	No	Yes- IPF	No
Qualitative findings of diffuse brain atrophy	No	No	Yes, mild	No	No	No	No	No	Yes, mild	Yes, mild	Yes, mild	Yes, mild
Other neuroimaging abnormalities	No	No	No	Yes- mild atrophy R optic nerve	Yes- wallerian degeneration R CST	No	No	Yes- PRES	Yes- PRES	No	No	Yes- hydromyelic cavity (D7-D9)
Number of available FU brain MRI	9	6	6	11	4	2	6	0	0	1	1	1
Age at last available brain MRI	19Y + 1mo	12Y + 2mo	13 Y	10Y + 11mo	18Y + 6mo	16Y + 11mo	26Y + 6mo	NA	NA	20Y	23Y + 8mo	24Y + 10mo
New ischemic lesions at FU - **Circulation** - **Location**	Yes A & P Bilateral periaqueductal, L mesencephalon, nucleocapsular region, L thalamus	No	No	Yes P R thalamus (2)	No A & P R semioval centrum, L pons, L inferior cerebellar peduncle	Yes Spinal Spinal cord (C4)	Yes A & P Periventricular region bilaterally, L inferior cerebellar peduncle	NA	NA	Yes P R midbrain	No	No
Perivascular enhancing tissue	Yes- IPF	No	Yes- IPF	Yes- IPF	No	No	NA	Yes- L crural cistern, L IAC	NA	No	No	No
Brain MRA/DSA/CTA findings	N	N	N	Reversible stenosis L PCoA	Two intracranial aneurysms	N	N	N	NA	N	N	N

					(branch of ACoA complex, L SCA)*							

Legend: A- Anterior, ACoA- Anterior communicating artery, CC- Corpus callosum, CST- Corticospinal tract, FU- Follow-up, GM- Gray matter, IAC- Internal auditory canal, IPF- Interpeduncular fossa, L- Left, mo- Months, N- Normal, no- Number, NA- Non applicable, P- Posterior, PCoA- Posterior communicating artery, PRES- Posterior reversible encephalopathy syndrome, R- Right, SAH- Subarachnoid acute hemorrhage, SCA- Superior cerebellar artery, Y- Years

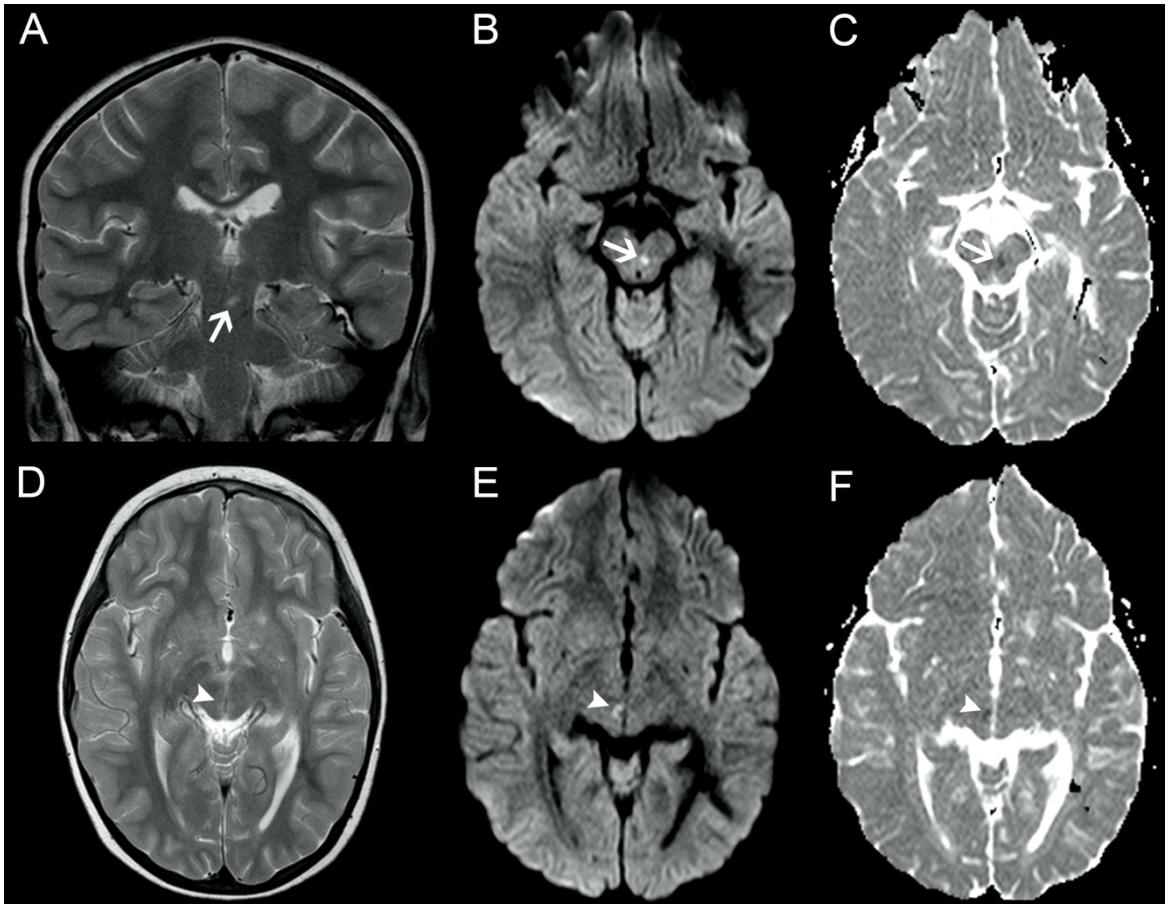
*Also history of a right middle meningeal artery ruptured aneurysm surgically treated during evacuation of a lobar hematoma

Online Figure Legends

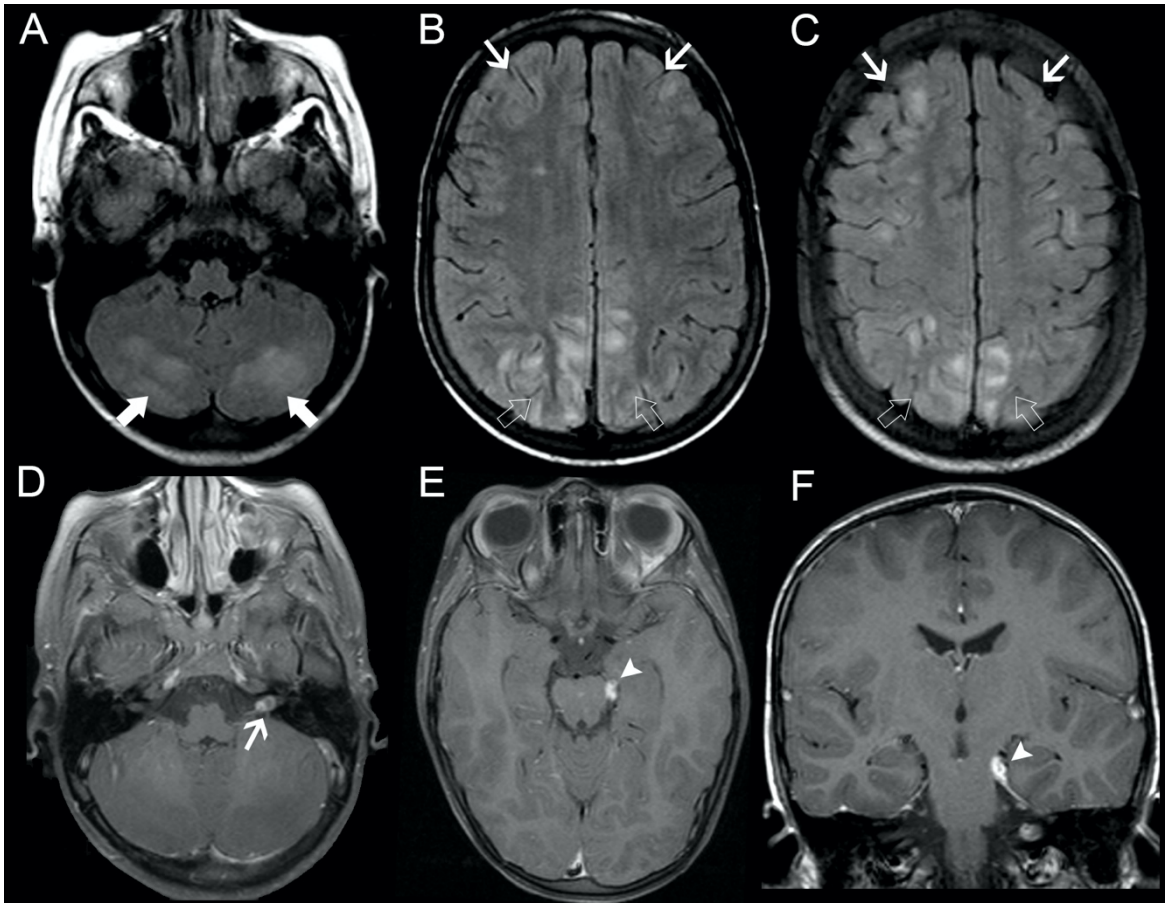


Online Fig 1. Chronic brain ischemic infarcts in three patients with adenosine deaminase 2 deficiency. Axial T2WI of patient #12 at 18.2 years of age (A,B) demonstrates multiple chronic lacunar ischemic infarcts distributed in both the anterior and posterior cerebral territories, namely in the right ponto-mesencephalic junction (arrow), left globus pallidus (arrowhead) and right thalamus

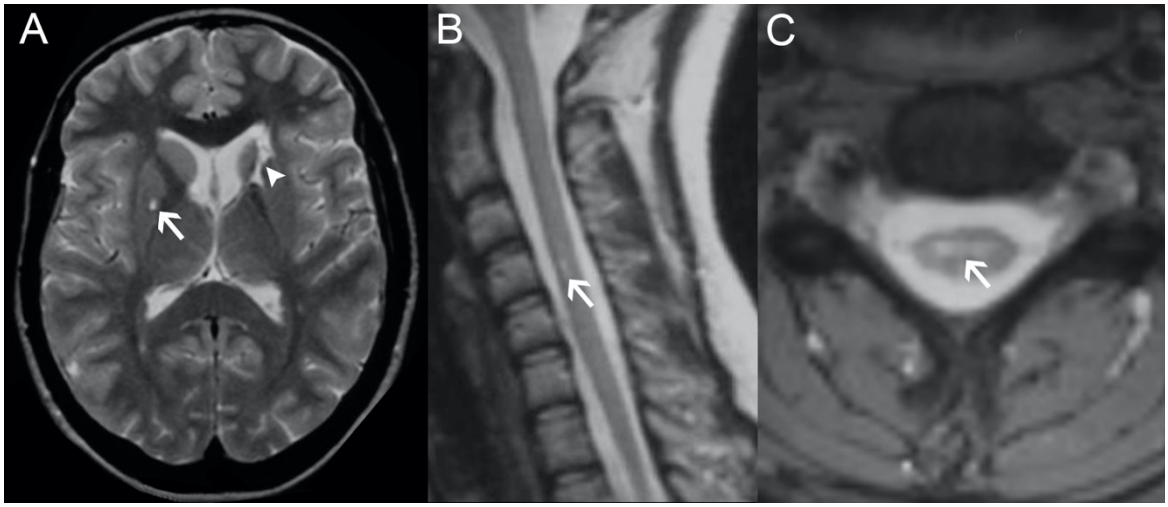
(open arrow). Axial (C) and coronal (D) T2WI of patient #3 at 6.2 years of age depicts multiple old lacunar infarcts in the left cerebral peduncle (arrows) and thalami bilaterally (arrowheads). Axial T2WI (E,F) of patient #10 at 19.9 years of age (D,E) shows chronic ischemic lesions in the right pontine protuberance (arrow) and cerebral peduncle (arrowhead).



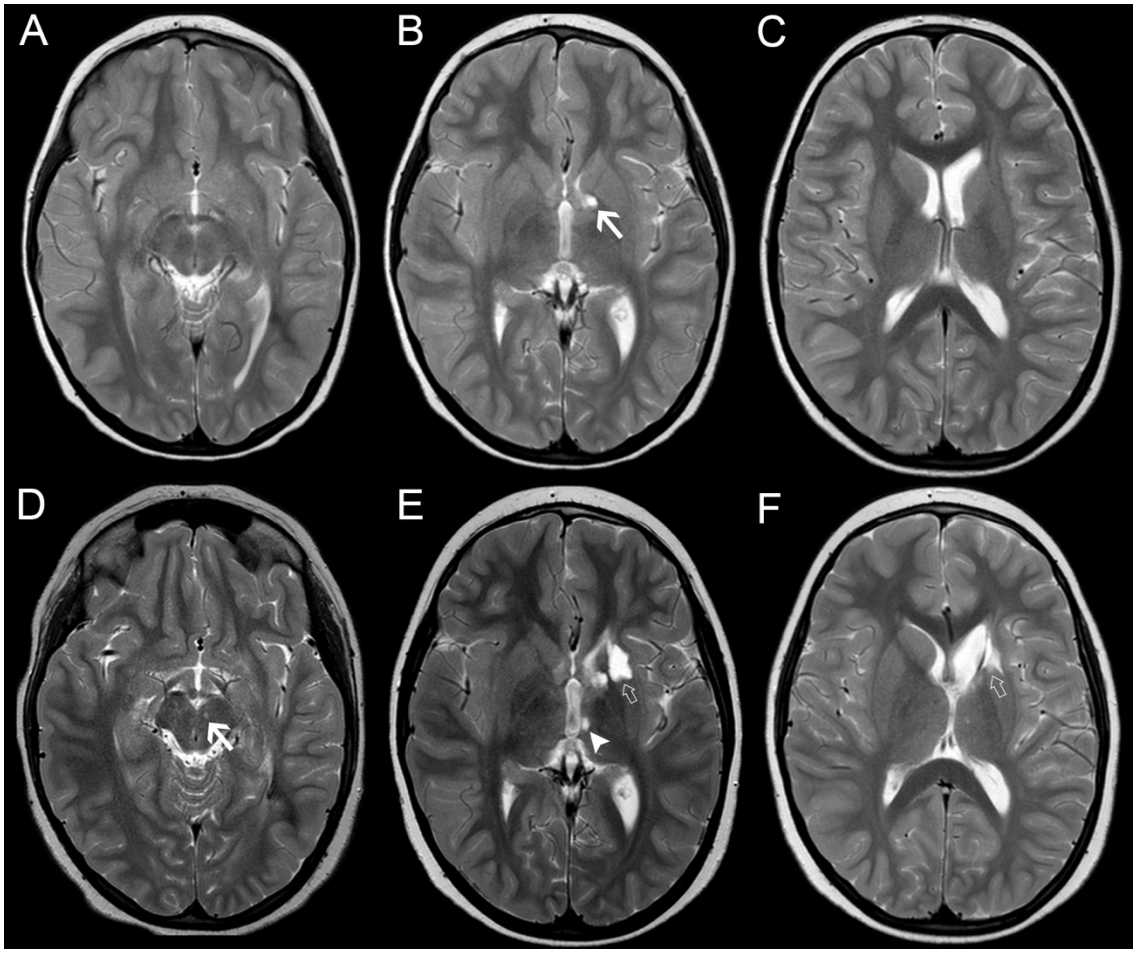
Online Fig 2. Acute brain ischemic infarcts in patient #1. Coronal (A) and axial (D) T2WI, axial DWI (B,E) and axial ADC maps (C,F) performed at 8.5 years of age detect two punctiform acute ischemic lesions in the posterior circulation territory, namely in the left mesencephalon (arrows) and right mesencephalic-diencephalic region (arrowheads). At that time, this patient also presented at that time a perivascular enhancing tissue in the interpeduncular fossa (See Fig 2).



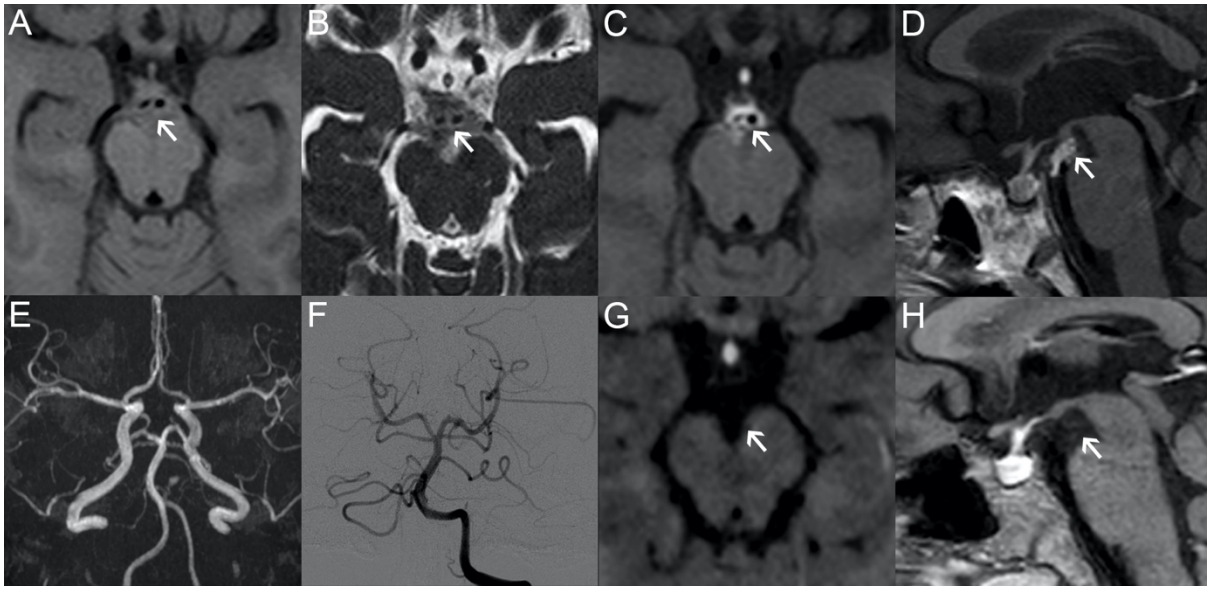
Online Fig 3. Posterior encephalopathy reversible syndrome and perivascular enhancing tissue in patient #8. Axial FLAIR images (A-C) performed at 7.6 years of age demonstrate bilateral asymmetric areas of hyperintensity involving the cerebellar hemispheres (thick arrows) as well as the anterior (arrows) and posterior (open arrows) brain watershed regions, in a pattern compatible with posterior reversible encephalopathy syndrome. Also note in the same patient extra-axial foci of enhancement in the inner auditory canal (arrow) and left crural cistern (arrowheads) on post-gadolinium axal fat sat T1WI (D,E) and post-gadolinium coronal TWI (F).



Online Fig 4. Chronic brain and medullary ischemic infarcts in patient #6. Brain axial T2WI (A) of patient #6 at 27 years of age depicts small chronic lacunar infarcts in the right putamen (arrow) and left anterior nucleo-capsular region (arrowhead). Sagittal T2WI (B) and axial T2*WI (C) of the cervical spine of the same patient show a focal hyperintense lesion involving the central gray matter at the level of C4 (arrows), in keeping with a focal ischemic lesion of the spinal cord.



Online Fig 5. Accrual of brain ischemic lesions over time in patient #1 before treatment with anti-TNF. Axial T2WI (A-C) at 8.1 years of age show one chronic ischemic lacunar lesion in the left globus pallidus (arrow). Axial T2WI (D-F) of the same patient at 9.7 years of age reveals three additional chronic ischemic lesions, namely in the left mesencephalon (arrow), thalamus (arrowhead) and anterior nucleocapsular region (open arrow).



Online Fig 6. Longitudinal neuroimaging of deficiency-related perivascular enhancing tissue in patient #4. Brain MRI performed at 5.8 years of age (A-D) demonstrates a soft-tissue component mass in the interpeduncular cistern surrounding the basilar artery and its terminal branches. This component is iso-mildly hyperintense on T1WI (A) (arrow) and its extension is better delineated on 3D driven equilibrium (DRIVE) images (B) (arrow). There is intense solid enhancement on post-gadolinium axial (C) and sagittal (D) T1WI images, with concentric vessel wall involvement (arrows). Time-of-flight MRA (E) and DSA (F) do not depict abnormal vessels in the corresponding location. Axial (G) and sagittal (H) black blood vessel wall imaging performed at 10.8 years of age after introduction of anti-TNF treatment, show complete resolution of this tissue (arrows).