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# A Family with a Novel CTLA4 Haploinsufficiency Mutation and Neurological Symptoms

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To the Editor,

CTLA4 haploinsufficiency is a rare autosomal dominant immune dysregulation disorder first described in 2014 [1, 2]. Patients with this disorder exhibit reduced expression of CTLA4, an inhibitory receptor that is found on activated and regulatory T lymphocytes, with subsequent T cell hyperactivation and lymphoproliferation. Here we report three members of the same family with a novel CTLA4 haploinsufficiency and neurological complications.

## Case 1

The index case presented with severe headaches at the age of 45. MRI brain revealed a focus of hypointensity in the frontal horn of the right lateral ventricle, which was stable on interval imaging. Past medical history and additional investigations are summarized in Table 1.

Family history (Suppl. Fig. 1) included two brothers with Evans syndrome (autoimmune thrombocytopenia and hemolytic anemia), a brother with unexplained lymphadenopathy, a brother who died at the age of 40 from left ventricular fibrosis,

and a niece with recurrent cutaneous ulceration who was carrier for an LRBA mutation. Her eldest daughter developed acute myeloid leukemia at age 14, and her other two children are discussed below (Cases 2 and 3).

At age 48, she developed left-sided numbness and weakness. Carotid ultrasound and echocardiogram were normal and the brain MRI scan was unchanged. A few months later, she presented with vertigo, vomiting, seizures, and right-facial dysesthesia, with dysarthria and right-sided dysmetria. Brain MRI now revealed a mass in the right middle cerebellar peduncle with surrounding edema (Fig. 1a). Cerebrospinal fluid (CSF) analysis demonstrated elevated protein and unmatched oligoclonal bands but no microorganisms. Symptoms responded to dexamethasone (16 mg daily) but relapsed on steroid wean (4 mg daily) when repeat MRI showed an enlarging mass. Histological analysis of the cerebellar peduncle mass was consistent with a florid active inflammatory and demyelinating process with neuronal sparing. The cellular infiltrate consisted of T cell lymphocytes, with a 2:1 ratio of CD4:CD8 T cells, plasma cells, and microglia [3]. Corticosteroids were restarted with clinical benefit, and a repeat MRI scan a few months later confirmed a radiological response (Fig. 1b).

Subsequent next generation sequencing of 194 genes associated with immune deficiency confirmed a heterozygous novel frameshift deletion in her CTLA4 gene, exon 1 (c.81dup encoding p.Leu28Serfs\*32). This was confirmed by Sanger sequencing. In combination with the clinical picture and family history, a diagnosis of CTLA4 haploinsufficiency was made. She was started on sirolimus and the cerebellar peduncle lesion resolved leaving a small focus of presumed gliosis at the site of biopsy (Fig. 1c). No interval change has

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**Table 1** Clinical features and immunological investigations of presented cases

<b>CLINICAL HISTORY</b>	
<b>Age</b>	<b>Case 1 (index case)</b>
35	Pernicious anaemia
43	Steroid responsive, episodic lymphadenopathy, fevers, myalgia, arthralgia; normal inflammatory markers; imaging confirmed fluctuating axillary, pelvic, inguinal lymphadenopathy, scattered opacification & small nodules in the lungs, mild bronchiectasis, borderline splenomegaly; lymph node biopsy: non-necrotising granulomas & multi-nucleate giant cell/ neutrophilic infiltrate
45	Erosive arthritis of left temporomandibular joint following bisphosphonate therapy
48	Lymph node biopsy: granulomatous lymphadenitis and suggestion of EBV-related lymphoid proliferation; serum EBV PCR negative
49	CT chest: deteriorating bronchiectasis, patchy airspace opacification, migratory infiltrates
50	MRI right leg: 1.4cm mass behind tibialis posterior tendon, spontaneous resolution
51	SeHCAT study: bile salt malabsorption
<b>Age</b>	<b>Case 2</b>
11	Autoimmune haemolytic anaemia; Café au lait spots; splenomegaly
12	Evan's syndrome
14	Splenectomy; low IgG
15	Liver biopsy: macronodular cirrhosis with active autoimmune hepatitis; fungal pneumonia; lung biopsy: bronchial associated lymphoid hyperplasia; arthralgia; normal imaging of affected joints
16	Pernicious anaemia; gastric biopsies: antral gastritis & intestinal metaplasia; treated with growth hormone & ethinyloestradiol for delayed puberty
21	Recurrent bacterial pneumonias ( <i>H. influenzae</i> , <i>Pseudomonas</i> , <i>Klebsiella</i> ); CT chest: generalised bronchial wall thickening, diffuse tree-in-bud infiltrates and mild cylindrical bronchiectasis
25	Duodenal biopsy: focal total villous atrophy with increased intraepithelial lymphocytes unresponsive to gluten withdrawal
<b>Age</b>	<b>Case 3</b>
17	Autoimmune hypothyroidism
19	Anosmia; normal nasoendoscopy and skull base on MRI
20	Autoimmune thrombocytopenia; iron deficiency anaemia; normal upper endoscopy
29	Type 1 diabetes; splenomegaly

Table 1 (Continued)

IMMUNOLOGICAL INVESTIGATIONS (serum)				
	Case 1	Case 2	Case 3	Reference range
<b>IgG</b>	7.5	4.1	8.7	6-16 g/l
<b>IgA</b>	1.7	0.3	2.2	0.8-2.8 g/l
<b>IgM</b>	1.6	0.7	1.5	0.5-1.9 g/l
<b>IgE</b>	<1	<1	ND	1-113 kU/L
<b>Pneumococcal serology</b> (post vaccination)	ND	2/12	ND	>7/12 serotypes expected
<b>C3</b>	1.36	0.7	1.4	0.8-2.1 g/l
<b>C4</b>	0.3	0.09	0.3	0.15-0.5 g/l
<b>CH50</b>	100%	100%	ND	>70%
<b>AP50</b>	100%	100%	ND	>70%
<b>T cells</b>	0.89 (81%)	0.54 (77%)	1.3 (78%)	0.67-3.04 *10 <sup>9</sup> /L
<b>CD4 T cells</b>	0.56 (51%)	0.48 (69%)	0.6 (36%)	0.38-1.84 *10 <sup>9</sup> /L
<b>CD8 T cells</b>	0.29 (26%)	0.06 (8%)	0.69 (42%)	0.31-1.6 *10 <sup>9</sup> /L
<b>NK cells</b>	0.14 (13%)	0.14 (20%)	0.13 (8%)	0.1-0.76 *10 <sup>9</sup> /L
<b>B cells</b>	0.07 (6%)	0.04 (5%)	0.22 (13%)	0.11-0.64 *10 <sup>9</sup> /L
<b>CD3+, TCR alpha/beta+, CD4/CD8- T cells</b>	1.8%	3.3%	ND	NP
<b>Lymphocyte proliferation</b> to PMA/ionomycin & PHA	100% of control	79% of control	ND	NP
<b>FasL (CD95) expression</b>	ND	normal	ND	NP
<b>NK cell perforin expression</b>	93% of control	80% of control	ND	NP
<b>TPO antibodies</b>	697	38	>1000	<100 IU/ml
<b>GAD antibodies</b>	negative	negative	>50000	<5 IU/ml
<b>TTG IgA, EMA IgG antibodies</b>	negative	negative	negative	negative
<b>ANA, dsDNA, ENA antibodies</b>	negative	negative	negative	negative
<b>AMA, LKM, SMA antibodies</b>	negative	negative	ND	negative
<b>Hu, Yo, Ri, PNMA2, CV2, amphiphysin antibodies</b>	negative	ND	negative	negative

Table 1 (Continued)

CSF RESULTS				
<b>Total protein</b>	0.75	ND	0.82	<0.54
<b>Glucose</b>	>2/3 of plasma	ND	>2/3 of plasma	>2/3 of plasma
<b>WBC</b>	4 /mm <sup>3</sup>	ND	7 /mm <sup>3</sup>	<1 /mm <sup>3</sup>
<b>MC&amp;S</b>	no growth	ND	no growth	no growth
<b>PCR for enterovirus, HSV1, HSV2, VZV, EBV, CMV, HHV6</b>	negative	ND	negative	negative
<b>IgG oligoclonal pattern</b>	present	ND	present	negative
<b>GAD antibodies</b>	ND	ND	16729	<10.9 IU/ml

ANA anti-nuclear antibodies, AMA anti-mitochondrial antibodies, ANCA anti-neutrophil-cytoplasmic antibodies, CMV cytomegalovirus, CSF cerebrospinal fluid, dsDNA double stranded DNA, EBV Epstein-Barr virus, EMA anti-endomysial antibodies, ENA extractable nuclear antigen, GAD glutamic acid decarboxylase, HHV6 human herpes virus 6, HSV1 herpes simplex virus 1, HSV2 herpes simplex virus 2, LKM liver kidney microsomal, MC&S microscopy, culture, and sensitivities, ND not done, NP not provided, PCR polymerase chain reaction, SMA anti-smooth muscle antibodies, TPO thyroid peroxidase, TTG tissue transglutaminase, VZV varicella zoster virus, WBC white blood cells

been seen on MRI over the following 3 years but the patient continues to experience focal seizures.

## Case 2

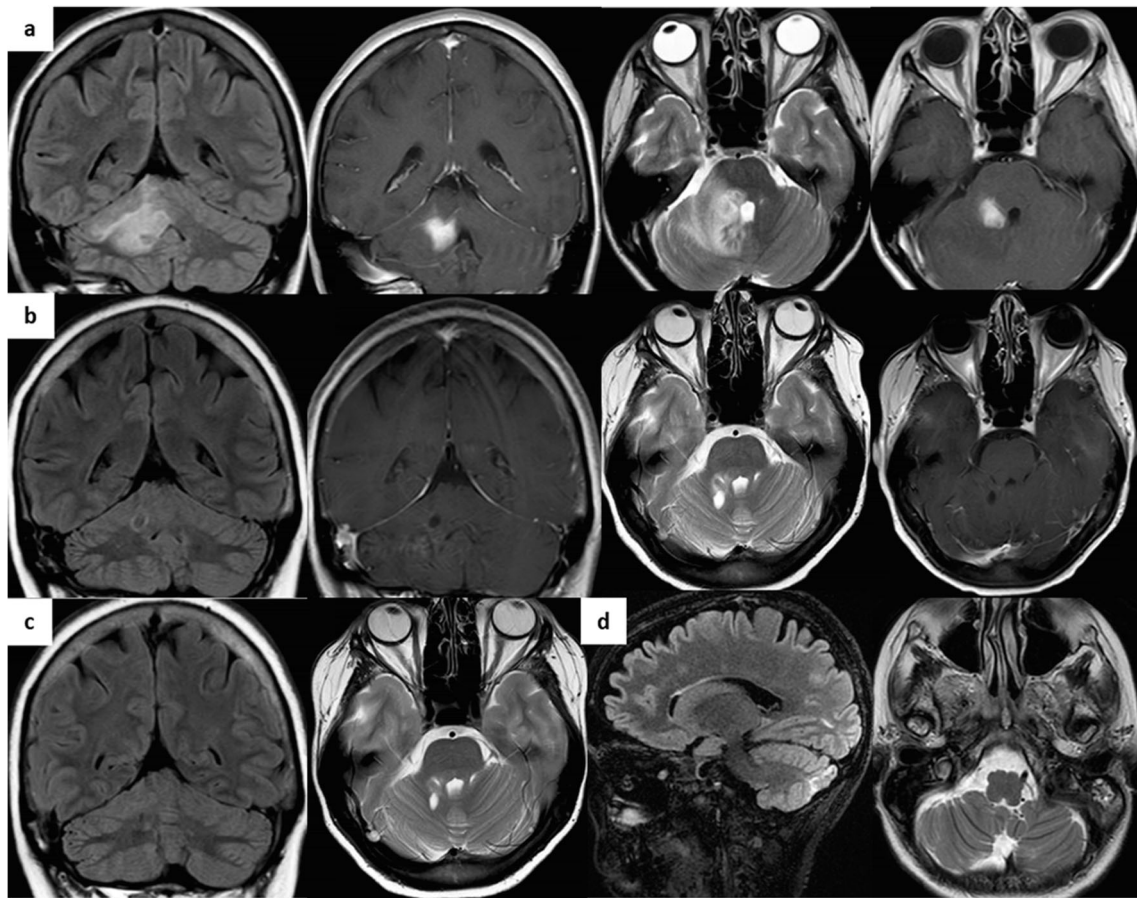
This patient was the second daughter of the index case. She had her first hospital admission at the age of 10 months, with suspected viral meningitis. Her subsequent clinical course is set out in Table 1. At the age of 12 years, she presented with severe headache, and brain MRI revealed a high signal intensity lesion in the right cerebellar hemisphere (Fig. 1d) and left superior frontal gyrus. She was treated with high-dose oral corticosteroids for presumed central nervous system (CNS) inflammation. At age 15 years, she presented with recurrent episodes of headaches, peripheral paresthesia, and muscle cramps and was again treated with corticosteroids.

She was referred to adult immunology at the age of 18 years, and investigations revealed two benign polymorphisms in her perforin gene. Genetics for autoimmune lymphoproliferative syndrome were however negative (Fas, Fas ligand, Caspase 10, Caspase 8, NRAS genes).

Following her mother's diagnosis of CTLA4 haploinsufficiency, she was confirmed to have the same genetic mutation. She is now on immunoglobulin replacement for hypogammaglobulinemia with corticosteroid and azathioprine to manage autoimmune hepatitis.

## Case 3

This 32-year-old male, son of the index case, has type I diabetes and hypothyroidism. From the age of 29 years, he developed headaches, nausea, memory impairment, poor coordination, as well as olfactory and auditory hallucinations. MRI brain scans, including venography, were normal and there was no enhancement with gadolinium. CSF analysis revealed a mild lymphocytosis with raised CSF protein (Table 1). Unmatched oligoclonal bands were detected on one occasion but were not persistent. Electroencephalography confirmed complex partial seizures arising from the right hemisphere and occurring on a background of mild excess of nonspecific slow and theta activity. Anti-N-methyl-d-aspartate receptor and voltage-gated potassium channel antibodies were negative, but anti-thyroid peroxidase (TPO) and glutamic acid decarboxylase (GAD) antibodies were detected. Subacute memory impairment, altered personality, and psychiatric



**Fig. 1** Brain MRI in cases 1 and 2. **a** Case 1 right middle cerebellar peduncular mass with surrounding edema (left, coronal views with and without gadolinium; right, axial views with and without gadolinium); **b** Case 1 reduction in the mass size post-treatment (left, coronal views with and without gadolinium; right, axial view with and without gadolinium).

**c** Case 1 further reduction in the mass size leaving a small focus of presumed gliosis (left, coronal view; right, axial view); **d** Case 2 right inferior cerebellar high signal intensity (left, sagittal view; right, axial view)

symptoms together with the emergence of seizures, CSF pleocytosis, and autoantibodies (anti-GAD) were in keeping with autoimmune encephalitis [4]. He was treated with corticosteroids, plasmapheresis, and anticonvulsant therapy with introduction of azathioprine and then mycophenolate as a steroid-sparing agent. Following the diagnosis of CTLA4 haploinsufficiency (age 32 years), he was switched to sirolimus with some improvement to his symptoms; headaches and hallucinations resolved, seizure control improved, and there has been no further decline in cognitive function.

## Discussion

Haploinsufficiency mutations in CTLA4 are known to be pathogenic. To date, 45 mutations have been described in the CTLA4 gene; 8 in exon 1, 31 in exon 2, and 6 in exon 3 (Suppl. Fig. 2). This family has a novel mutation, involving duplication of a nucleotide in exon 1 of the CTLA4 gene

(cDNA position 81), with a predicted amino acid change of Leu28Serfs\*32. This results in a cDNA frameshift and the introduction of a stop codon a short distance downstream, with significantly truncated CTLA4 protein.

A heterozygous variant of uncertain significance in LRBA exon 42 c.6424 T > C, encoding p. Phe2142Leu, was also detected in the index case and in her niece (patient with recurrent cutaneous ulceration; Supplementary Fig. 1). Heterozygous LRBA mutations are not known to be pathogenic and indeed LRBA protein expression was found to be normal in both patients. Another brother has recently presented with seizures and steroid-responsive CNS infiltration; genetic testing confirms the familial CTLA4 mutation (Supplementary Fig. 1).

These cases illustrate phenotypic variability associated with CTLA4 haploinsufficiency. Symptoms have been reported from infancy to adulthood, with a median age of 11 years [5]. Lymphoproliferation is the most common manifestation (73%), followed by autoimmune cytopenias (62%), and

respiratory (68%) and gastrointestinal (59%) symptoms [5]. Neurological complications have been reported in 28% of patients [5], and histologically confirmed CNS inflammation has been previously described [6].

Although the role of CTLA4 in the CNS is unknown, reduced CTLA4 function is expected to lead to neuroinflammation. Interestingly, patients with multiple sclerosis (MS) have reduced CTLA4 expression [7], while certain CTLA4 polymorphisms are linked to reduced remyelination in MS [8]. Encephalitis and demyelination have also been reported in patients treated with ipilimumab [9, 10], a CTLA4-blocking monoclonal antibody used in the treatment of melanoma. Lymphoproliferation with demyelination and mass effect largely explain the observed neurological features in the index case. The etiopathogenesis in case 3 could be autoimmune; e.g., anti-GAD antibodies are known to be associated with limbic encephalitis, stiff person syndrome, and ataxia.

Overall, these cases highlight varied neurological sequelae associated with CTLA4 haploinsufficiency beyond lymphocytic infiltration, to include autoimmune-mediated damage within the CNS.

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**Availability of Data and Materials** Not applicable.

**Code Availability** Not applicable.

## Declarations

**Conflict of Interest** The authors declare no competing interests.

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