NEUROMUSCULAR DISORDERS AND PERIPHERAL NEUROPATHIES - CASE REPORT COLLECTION 2021

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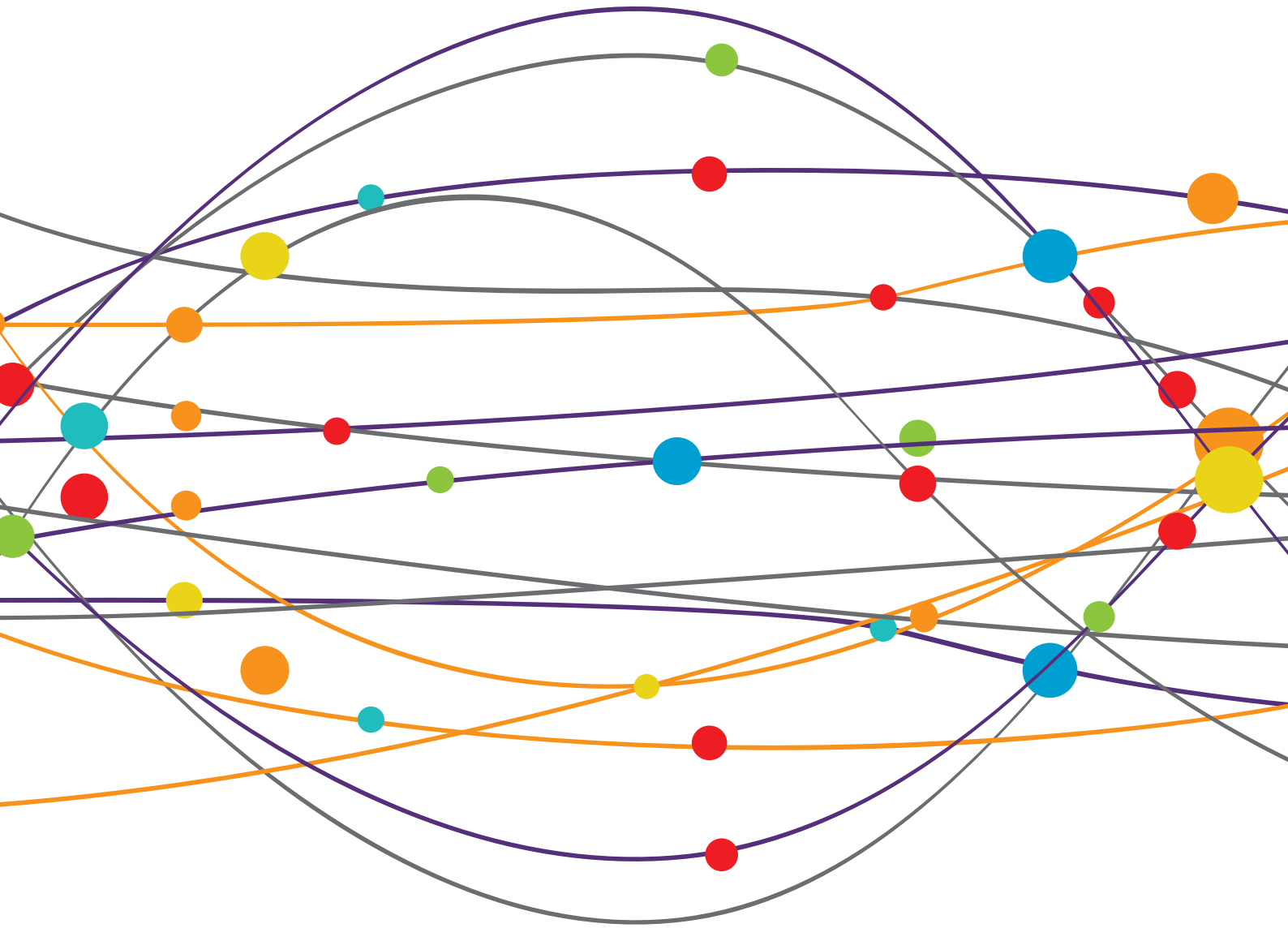

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NEUROMUSCULAR DISORDERS AND PERIPHERAL NEUROPATHIES - CASE REPORT COLLECTION 2021

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CASE REPORT
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Pembrolizumab-Induced Isolated Cranial Neuropathy: A Rare Case Report and Review of Literature

Francesco Bruno[1], Rosa Antonietta Palmiero[1], Bruno Ferrero[2], Federica Franchino[1], Alessia Pellerino[1], Enrica Milanesi[3], Riccardo Soffietti[1] and Roberta Rudà[4]*

[1] Department of Neuro Oncology, University Hospital of the City of Health and Science of Turin, Turin, Italy, [2] Department of Neurology, University Hospital of the City of Health and Science of Turin, Turin, Italy, [3] Department of Oncology, University Hospital of the City of Health and Science of Turin, Turin, Italy, [4] Department of Neurology, Castelfranco Veneto Hospital, Castelfranco Veneto, Italy

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Edited by:
Chiara Briani,
University of Padua, Italy

Reviewed by:
Paola Sandroni,
Mayo Clinic, United States
Roser Velasco,
Catalan Institute of Oncology, Spain

****Correspondence:***
Francesco Bruno
f.bruno@unito.it

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Introduction: Anti-PD1 agents are widely used in the treatment of solid tumors. This has prompted the recognition of a class of immune-related adverse events (irAEs), due to the activation of autoimmune T-cells. Pembrolizumab is an anti-PD1 agent, which has been related to an increased risk of various neurological irAE (n-irAEs). Here, we present a rare case of pembrolizumab-induced neuropathy of cranial nerves.

Case Report: A 72-year-old patient was diagnosed with a lung adenocarcinoma in February 2018 (EGFR–, ALK–, and PDL1 90%). According to the molecular profile, pembrolizumab was started. After three administrations, the patient developed facial paresis, ptosis, ophthalmoplegia, and dysphonia. As brain metastases and paraneoplastic markers were excluded, a drug-related disorder was suspected and pembrolizumab was discontinued. A nerve conduction study and electromyography excluded signs of neuropathy and myopathy at four limbs, and repetitive nerve stimulation was negative. However, altered blink reflex and nerve facial conduction were consistent with an acute neuropathy of the cranial district. Thus, the patient was treated with two cycles of intravenous immunoglobulins (IVIg), which rapidly allowed improvement of both symptoms and neurophysiological parameters. However, the patient died in October 2018 for a progression of lung tumor.

Discussion: Only 16 cases of pembrolizumab-related neuropathies have been described so far. Our case is of particular interest for the isolated involvement of cranial nerves and the prompt response to IVIg.

Conclusion: N-irAEs are insidious conditions that require solid knowledge of onco-immunotherapy complications: it is mandatory not to delay any treatment that would potentially modify the course of a neurological complication.

Keywords: pembrolizumab, anti-PD1 agents, neurological immune-related adverse effects, immune-related neurological complications, autoimmune neuropathy, case report

INTRODUCTION

Pembrolizumab (an anti-PD1 agent) may favorably impact the outcome of melanoma and non-small cell lung carcinoma (NSMLC) (1, 2). By promoting the activation of T-cells, pembrolizumab fosters the immune response against tumor. However, it may also increase the risk of autoimmune reactions, known as immune-related adverse events (irAEs). Various neurological irAEs (n-irAEs) have been associated with pembrolizumab: in clinical trials with checkpoint inhibitors, 6.3% of patients on pembrolizumab presented n-irAEs of any type and grade (3). The peripheral nervous system is more likely to be involved than the central nervous system (4). In a recent systematic review focused on pembrolizumab-induced neuromuscular disorders (5), 14 (36%), 13 (33%), 9 (23%), and 3 (8%) of 39 patients on pembrolizumab were reported to develop myopathy, myasthenia gravis, neuropathy, or overlapping disorders, respectively.

Here, we describe a patient who developed a rare acute neuropathy of cranial nerves from pembrolizumab.

CASE REPORT

In February 2018, a 72-year-old man was diagnosed with an adenocarcinoma of the lung (EGFR–, ALK–, and PDL1 amplificated in 90% of the cells). A total-body CT scan and an FDG-PET ruled out the presence of metastases at presentation. Based on the molecular profile, pembrolizumab was started. After three cycles (June 2018), the patient developed fatigue, dizziness, mild bilateral facial palsy (grade III of the House–Brackmann scale), bilateral ptosis and ophthalmoplegia, dysphonia, and dysphagia. As the brain and spine MRI with gadolinium excluded the occurrence of metastases, a neuroimmunological drug-related disorder or a paraneoplastic syndrome was considered, and pembrolizumab was stopped. First, we ruled out the presence of neuromuscular junction disorders: both repetitive nerve stimulation (RNS) and specific antibody assays—including anti-acetylcholine receptor (AChR), anti-muscle-specific kinase (MuSK), and P/Q-type VGCC antibodies—were negative. Second, we tested the markers of immune-mediated neuropathy (anti-MAG, anti-GM1/2, anti-GD1a/b, and anti-GQ1b antibodies) and paraneoplastic syndromes (anti-Tr, anti-CV2/CRMP5, anti-amphiphysin, anti-PNMA2/TaMa, anti-GAD65, anti-recoverin, anti-Ri, anti-Yo, anti-Hu, anti-Zic4, anti-SOX1, and anti-titin antibodies), with negative results. Also, creatine kinase was normal (80 IU/l), and cerebrospinal fluid (CSF) did not harbor any inflammatory alterations (being cell count 5/mm^3 and protein concentration 0.32 g/l). Then, we performed nerve conduction studies (NCS) and electromyography (EMG) at the limbs and cranial district: while no signs of neuropathy or myopathy were seen at the extremities, the evidence of slightly decreased amplitude of facial nerve conduction and altered blink reflex (lacking both ipsilateral and contralateral R2 components) suggested a diagnosis of a neuropathy involving the cranial nerves (**Tables 1A,B**). Therefore, in July 2018, the patient was treated with intravenous immunoglobulins (IVIg: 0.4 g/kg/5 days), with no use of oral glucocorticoids due to the presence of moderate dysphagia. The therapy was well-tolerated and allowed a prompt relief from dizziness, diplopia, and dysphonia and total remission of facial palsy. Also, NCS of the facial nerves and blink reflex showed a rapid improvement, as both ipsilateral and contralateral R2 components were almost completely restored after the first cycle of IVIg (**Tables 1A,B**). Due to the rapid improvement of symptoms, the employment of intravenous steroids was not needed, but a second cycle of IVIg was administered in August 2018 to consolidate the result. The neurological condition remained stable until October 2018, when the patient died for a progression of the primary tumor.

We identified 24 cases of pembrolizumab-induced neuropathies and/or radiculopathies, mostly reported in small series of single or few patients (**Table 2**) (6–18). Melanoma was the primary tumor in 20 cases, whereas only three patients had lung adenocarcinoma (6, 7, 16). Fourteen patients were treated with pembrolizumab as a single agent (three of them had been previously treated with ipilimumab), three with a combination of pembrolizumab with chemotherapy, and seven with an association of pembrolizumab and ipilimumab. Seven patients developed acute demyelinating polyradiculopathy involving the lower and upper extremities, thus mimicking Guillain-Barré syndrome (GBS); three presented involvement of both limbs and cranial district, similar to GBS—Miller Fisher variant; and only five isolated neuropathies of cranial nerves were described (8, 18). Immune-mediated neuropathy occurred with a median latency of four cycles. The diagnostic workup included nerve conduction studies (NCS) and electromyography (EMG) in 18 patients, lumbar puncture in 14 patients, and dosage of serum antibodies of autoimmune neuropathies or paraneoplastic syndromes in eight cases. CSF harbored albuminocytologic dissociation in five cases (7, 10, 11, 14), while in seven cases, it showed pleocytosis (7, 10, 12, 13, 17, 18), and in two cases, it was normal (18).

DISCUSSION

Pembrolizumab-induced acute neuropathy is a rare n-irAE. It is not clear whether the association with other checkpoint inhibitors could drive synergically the onset of the condition. Based on our review of literature, neurological symptoms, such as limb weakness and/or sensory disorders, as well as brainstem and cranial nerve deficits, usually appear soon after the initiation of pembrolizumab and should be carefully investigated in order to rule out differential diagnoses, especially CNS metastases or paraneoplastic syndromes. MRI of the brain and the spine, CSF analysis, neurophysiological studies, and laboratory tests for autoimmune neuropathies and paraneoplastic syndromes are the most useful procedures for the diagnosis. For instance, in cases of polyradiculopathy, MRI of the spine with gadolinium may reveal root enhancement, although this is not a regular finding: in a recent series (16), only two of five cases with facial neuropathies and four of six cases with polyradiculoneuropathy demonstrated gadolinium enhancement of cranial nerves or spinal nerve roots, respectively. CSF may be normal in a minority of cases, whereas

TABLE 1A | Nerve conduction study of the facial nerves at presentation, after the first cycle of IVIg, and after the second cycle.

Nerve	At presentation		After 1st cycle of IVIg		After 2nd cycle of IVIg	
	Latency onset ms	Amplitude mV	Latency onset Ms	Amplitude mV	Latency onset ms	Amplitude mV
Left n. facialis						
Mandible—orbicularis oculi	2.26	2.1	3.2	2.8	2.5	3.4
Mandible—nasalis	3.04	1.57	2.96	2.2	2.11	2.5
Right n. facialis						
Mandible—orbicularis oculi	2.65	1.79	3.28	3.3	2.81	3.3
Mandible—nasalis	3.82	2.1	3.04	2.3	3.74	2.3

Compound muscle action potential (CMAP) amplitude increased to normal values since after the first cycle of therapy.

TABLE 1B | Latencies of R1 and R2 components of blink reflex at presentation, after the first cycle of treatment, and after the second cycle.

Stimulation	Registration	At presentation				After 1st cycle of IVIg				After 2nd cycle IVIg			
		R1-latency		R2-latency		R1-latency		R2-latency		R1-latency		R2-latency	
		ms	RefDev	ms	RefDev	ms	RefDev	Ms	RefDev	ms	RefDev	ms	RefDev
Left	Left	12.0	1.85	A	NA	11.5	1.24	45.7	4.5	12.1	1.93	44.6	4.1
	Right			A	NA			48.6	5.3			43.6	3.9
	Difference			NA	NA			−2.9	−2.7			0.96	3.9
Right	Right	11.8	1.6	A	NA	11.8	1.60	39.6	2.7	11.1	0.75	38.1	2.2
	Left			A	NA			44.9	4.2			37.0	1.92
	Difference			NA	NA			−5.2	−4			1.07	−0.31

Reference values for NCS n. facialis: at orbicularis oculi, latency ≤3.1 ms, amplitude ≥1.0 mV; at nasalis, latency ≤4.2 ms, amplitude ≥1.0 mV. Reference values for blink reflex: R1 (ipsilateral), latency ≤13 ms, difference ≤1.2 ms; R2 (ipsilateral), latency ≤41 ms, difference ≤5 ms; R2 (contralateral), latency ≤44 ms, difference ≤7 ms.
A, absent; NA, not applicable; RefDev, deviation from reference.

it usually harbors some abnormalities, such as pleocytosis with or without increased protein level (19) or albuminocytologic dissociation; the prevalence and clinical meaning of autoimmune antibodies, which may be common in n-irAE affecting the CNS [as recently reported by Sechi et al. (20)], are not clearly determined so far; finally, although data provided by different authors are heterogeneous, NCS and EMG seem to have a higher sensitivity and specificity than laboratory tests.

We described a peculiar case of a lung adenocarcinoma patient who developed an acute neuropathy of cranial nerves from pembrolizumab. Cranial nerve disorders may be observed among patients developing neuropathies from checkpoint inhibitors, as reported by Dubey et al. (16): in this series, seven out of 19 patients with peripheral n-irAEs showed cranial nerve involvement, with or without meningitis, and six had non-length-dependent polyradiculopathies with or without cranial nerve disorders. However, as far as we know, only five cases of pembrolizumab-induced isolated neuropathy of cranial nerves have been described so far: all those cases are described in melanoma patients (8, 18), while only three patients with lung adenocarcinoma have been reported, and none of them presented an exclusive involvement of cranial nerves as in our case. Whether the over-representation of melanoma patients might just reflect the larger employment of pembrolizumab in this tumor or there is a causative correlation should be investigated in further studies. Our patient shares some features with other cases reported in literature: he presented with immune-related neuropathy only after three cycles of pembrolizumab; dismissal of the drug produced clinical benefits; and antibodies of autoimmune neuropathies were not detected by laboratory tests. However, he also presented peculiar features. First, CSF analysis did not show albuminocytologic dissociation or slight pleocytosis, as commonly seen in similar cases; second, he developed a multineuropathy of cranial nerves with no involvement of the extremities; furthermore, he presented with a complex disorder of multiple nerves: in fact, symptoms and signs due to the involvement of the III, IV, VI, VII, IX, and X nerves were all present, and NCS and altered blink reflex confirmed the damage of facial nerves and revealed a subclinical impairment of the trigeminal nerves. Conversely, in other cases of isolated pembrolizumab-derived cranial nerve disorders, patients have been usually reported to have mononeuropathies (mostly facial palsies) or involvement of few cranial nerves (18). Finally, IVIg therapy (not associated to steroids) dramatically impacted the clinical course of the disease, with an improvement of both symptoms and neurophysiological tests since the first cycle: in literature, only two patients were treated with IVIg alone (9, 10), while the most common

TABLE 2 | Pembrolizumab-induced neuropathy: review of literature.

Author	Patients	Cancer diagnosis	Treatment	Cycles to onset of n-irAE	Neurological presentation		Diagnostic workup				Diagnosis	Pembrolizumab stopped	Management of the n-irAE	Outcome (of the n-irAE)
					Limb weakness and/or sensory disorder	Cranial nerve involvement	CSF		NCS/EMG/ evoked potentials	Autoimmune antibodies	Exclusion of paraneoplastic syndrome			
Aya et al. (6)	1	Melanoma	Pembrolizumab (previous treatments: IFN-alpha, dacarbazine, and ipilimumab)	1	Yes	Yes (palsy of the abducens nerve)	NA		Sensory peripheral polyneuropathy	NA	NA	Vasculitic neuropathy (confirmed by nerve and muscle biopsy)	Yes	Oral and intravenous glucocorticoids	Improved
de Maleissye et al. (7)	2	Melanoma	Pembrolizumab	2	Yes	Yes (facial palsy)	Pleocytosis (45 cells/mm^3), slight increase of proteins (0.56 g/l)	No A-C dissociation	Demyelinating polyradiculopathy	NA	Yes	GBS, Miller-Fisher variant	Yes	IVIg	Improved
	3	Melanoma	Ipilimumab + pembrolizumab	6	Yes	No	Normal cells count; slight increase of proteins (0.74 g/l)	A-C dissociation	Demyelinating polyradiculopathy	NA	Yes	CIDP	Yes	Oral and intravenous glucocorticoids + PEX	Not improved
Zimmer et al. (8)	4	Melanoma	Pembrolizumab (previous treatments: IFN-alpha, dacarbazine, and ipilimumab)	4†	NA	Yes (paresis of the oculomotor nerve)	NA		NA	NA	NA	Neuritis of the oculomotor nerve	Yes	Prednisolone	Improved
	5	Melanoma	Pembrolizumab (previous treatments: IL2, dabrafenib/trametinib and ipilimumab)	11†	Yes	No	NA		NA	NA	NA	GBS	Yes	Prednisolone	Improved
Diamantopoulos et al. (9)	6	Melanoma	Pembrolizumab	1	Yes	No	NA		Axonal polyneuropathy and myositis	Ab anti-neuronal antigens - Ab anti-gangliosides - Ab related to myositis -	Yes	Overlapping axonal polyneuropathy and myositis	Yes	Methylprednisolone + IVIg + PEX	Deceased
Kao et al. (10)	7	Melanoma	Pembrolizumab	10	Yes	No	Normal cell count (2 cells/mm^3); slight increase of proteins (0.71 g/l)	A-C dissociation	Demyelinating polyradiculopathy	Ab anti-GM1 - Ab anti-GD1b -	Yes	GBS	Yes	Prednisone + IVIg	Improved
	8	Melanoma	Pembrolizumab	6	Yes	No	NA		Mixed axonal and demyelinating polyneuropathy	NA	NA	Peripheral mixed demyelinating and axonal neuropathy	Yes	Prednisone	Improved
	9	Melanoma	Pembrolizumab	20	Yes	Yes (facial palsy, dysphonia)	Pleocytosis (12 cells/mm^3); slight increase of proteins (0.95 g/l)	No A-C dissociation	Demyelinating polyradiculopathy	Ab anti-GM1/2 - Ab anti-GD1a/b - Ab anti-GQ1b -	Yes	GBS, Miller-Fisher variant	Yes	IVIg	Improved

(Continued)

TABLE 2 | Continued

Author	Patients	Cancer diagnosis	Treatment	Cycles to onset of n-irAE	Neurological presentation		Diagnostic workup					Diagnosis	Pembrolizumab stopped	Management of the n-irAE	Outcome (of the n-irAE)
					Limb weakness and/or sensory disorder	Cranial nerve involvement	CSF		NCS/EMG/ evoked potentials	Autoimmune antibodies	Exclusion of paraneoplastic syndrome				
Sepúlveda et al. (11)	10	Melanoma	Ipilimumab + pembrolizumab	23	Yes	No	No cells; slight increase of proteins (0.67 g/l)	A-C dissociation	Axonal polyradiculopathy	Ab anti-neuronal antigens - Ab anti-gangliosides -	Yes	GBS, AMAN variant	Yes	IVIg + PEX	Improved
Yost et al. (12)	11	Melanoma	Ipilimumab + pembrolizumab	3 months after pembrolizumab dismissal‡	No	Yes (facial palsy, dysphonia)	Pleocytosis (12 cells/mm^3); high proteins level (1.95 g/l)	No A-C dissociation	Altered blink reflex (absent R1/R2 responses)	Ab anti-GM1/2 - Ab anti-GD1a/b - Ab anti-GQ1b -	Yes	Isolate acute neuropathy of facial nerve	Yes	Methylprednisolone + IVIg	Improved
Fellner et al. (13)	12	Melanoma	Pembrolizumab	18 weeks after first pembrolizumab administration‡	Yes	No	Pleocytosis (58 cells/mm^3); high proteins level (2.27 g/l)	No A-C dissociation	Demyelinating polyradiculopathy	Ab anti-GD1b - Ab anti-GQ1b - Ab anti-MAG Ab anti-neuronal antigens -	Yes	GBS	Yes	Methylprednisolone	Improved
Manam et al. (14)	13	Lung adenocarcinoma	Pembrolizumab + carboplatin and pemetrexel	2	Yes	No	Slight increase of proteins (0.68 g/l); no cell count reported.	A-C dissociation (as reported by authors)	NA	NA	Yes	GBS	Yes	Methylprednisolone + IVIg + PEX	Improved
	14	Melanoma	Pembrolizumab + dabrafenib and trametinib	2	Yes	No	Slight increase of proteins (0.56 g/l); no cell count reported.	A-C dissociation (as reported by authors)	Demyelinating polyradiculopathy	Ab anti-GM1 -	Yes	GBS	Yes	PEX	Deceased (due to the n-irAE)
Ong et al. (15)	15	Lung adenocarcinoma	Pembrolizumab	2	Yes	Yes (facial palsy)	NA		Demyelinating polyradiculopathy	NA	Yes	GBS, Miller-Fisher variant	Yes	Methylprednisolone + IVIg	Improved
Dubey et al. (16)§	16	NA	Ipilimumab + pembrolizumab	1	NA	Yes (bilateral facial palsy)	NA		NA	NA	NA	Bilateral acute neuropathy of facial nerves	NA	NA	NA
	17	Melanoma	Pembrolizumab	2	Yes	No	NA		Lumbosacral radiculopathy and peripheral sensory neuropathy	NA	NA	GBS	Yes	None	Improved
	18	Melanoma	Pembrolizumab	1	Yes	No	NA		Length-dependent sensory and motor axonal polyneuropathy	NA	NA	Acute sensory and motor axonal polyneuropathy	No	Gabapentin 100 mg twice a day	Improved
	19	Lung adenocarcinoma	Erlotinib + pembrolizumab	1	Yes	No	NA		Multiple proximal mononeuropathy of left upper arm	NA	NA	Neuralgic amyotrophy	Yes	Prednisone 60 mg daily	Improved

(Continued)

TABLE 2 | Continued

Author	Patients	Cancer diagnosis	Treatment	Cycles to onset of n-irAE	Neurological presentation		Diagnostic workup					Diagnosis	Pembrolizumab stopped	Management of the n-irAE	Outcome (of the n-irAE)
					Limb weakness and/or sensory disorder	Cranial nerve involvement	CSF		NCS/EMG/ evoked potentials	Autoimmune antibodies	Exclusion of paraneoplastic syndrome				
Muralikrishnan et al. (17)	20	Melanoma	Pembrolizumab	2	Yes	No	Pleocytosis (17 cells/mm^3); slight increase of proteins (0.78 g/l)	No A-C dissociation	Demyelinating polyradiculopathy	Ab anti-gangliosides - Ab anti-MAG -	NA	GBS	Yes	Methylprednisolone + IVIg + PEX	Improved
Vogrig et al. (18)	21	Melanoma	Pembrolizumab	1	No	Yes (visual loss)	Pleocytosis (34 cells/mm^3), normal protein content	No A-C dissociation	NA	NA	NA	Optic neuropathy	Yes	None	Improved
	22	Melanoma	Ipilimumab + pembrolizumab	6 months after pembrolizumab initiation‡	No	Yes (visual loss)	Normal	No A-C dissociation	Altered visual evoked potentials (VEPs)	NA	NA	Optic neuropathy	Yes	Methylprednisolone	Not improved
	23	Melanoma	Ipilimumab + pembrolizumab	NA	No	Yes (visual / hearing loss)	Normal	No A-C dissociation	Altered visual evoked potentials (VEPs)	NA	NA	Optic neuropathy / auditory neuropathy	Yes	Methylprednisolone + PEX	Not improved
	24	Melanoma	Ipilimumab + pembrolizumab	1 month after pembrolizumab initiation‡	No	Yes (palsy of the abducens nerve)	Mild pleocytosis (6 cells/mm^3), normal protein content	No A-C dissociation	NA	NA	NA	Abducens nerve neuropathy	Yes	Oral glucocorticoids	Improved

†*The authors reported "13 weeks after first pembrolizumab administration": it would indicate four and 11 cycles for patients 5 and 6, respectively, as pembrolizumab was administered every 3 weeks, according to authors' note.*

‡*No exact number of cycles has been provided by the authors.*

§*A fifth case of a patient undergoing ipilimumab and pembrolizumab who developed an immune-related neuropathy is mentioned, but not described in the paper.*

Ab, antibodies; A-C dissociation, albuminocytologic dissociation; GBS, Guillain-Barré syndrome; IFN-alpha, interferon-alpha; IL2, interleukin 2; IVIg, intravenous immunoglobulins; NA, not applicable; PEX, plasma exchange.

strategies were a combination of steroids, plasma exchange, and/or IVIg.

CONCLUSION

In case of immune-mediated neuropathy, pembrolizumab should be dismissed immediately. According to our experience, IVIg can be a useful and effective treatment: nevertheless, a combination of steroids and/or plasma exchange should be considered based on clinical severity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.669493/full#supplementary-material

REFERENCES

1. Wang L, Ma Q, Yao R, Liu J. Current status and development of anti-PD-1/PD-L1 immunotherapy for lung cancer. *Int Immunopharmacol*. (2020) 79:106088. doi: 10.1016/j.intimp.2019.106088
2. Simeone E, Grimaldi AM, Festino L, Trojaniello C, Vitale MG, Vanella V, et al. Immunotherapy in metastatic melanoma: a novel scenario of new toxicities and their management. *Melanoma Manag*. (2019) 6:MMT30. doi: 10.2217/mmt-2019-0005
3. Cuzzubbo S, Javeri F, Tissier M, Roumi A, Barlog C, Doridam J, et al. Neurological adverse events associated with immune checkpoint inhibitors: review of the literature. *Eur J Cancer*. (2017) 73:1–8. doi: 10.1016/j.ejca.2016.12.001
4. Bruna J, Argyriou AA, Anastopoulou GG, Alemany M, Nadal E, Kalofonou F, et al. Incidence and characteristics of neurotoxicity in immune checkpoint inhibitors with focus on neuromuscular events: experience beyond the clinical trials. *J Peripheral Nerv Syst*. (2020) 25:171–7. doi: 10.1111/jns.12371
5. Johansen A, Christensen SJ, Scheie D, Højgaard JLS, Kondziella D. Neuromuscular adverse events associated with anti-PD-1 monoclonal antibodies. *Neurology*. (2019) 92:663. doi: 10.1212/WNL.0000000000007235
6. Aya F, Ruiz-Esquide V, Viladot M, Font C, Prieto-Gonzàlez S, Prat A, et al. Vasculitic neuropathy induced by pembrolizumab. *Ann Oncol*. (2017) 28:433–4. doi: 10.1093/annonc/mdw613
7. de Maleissye MF, Nicolas G, Saiag P. Pembrolizumab-induced demyelinating polyradiculoneuropathy. *N Engl J Med*. (2016) 375:296–7. doi: 10.1056/NEJMc1515584
8. Zimmer L, Goldinger SM, Hofmann L, Loquai C, Ugurel S, Thomas I, et al. Neurological, respiratory, musculoskeletal, cardiac and ocular side-effects of anti-PD-1 therapy. *Eur J Cancer*. (2016) 60:210–25. doi: 10.1016/j.ejca.2016.02.024
9. Diamantopoulos PT, Tsatsou K, Benopoulou O, Anastasopoulou A, Gogas H. Inflammatory myopathy and axonal neuropathy in a patient with melanoma following pembrolizumab treatment. *J Immunother*. (2017) 40:221–3. doi: 10.1097/CJI.0000000000000172
10. Kao JC, Liao B, Markovic SN, Klein CJ, Naddaf E, Staff NP, et al. Neurological complications associated with anti–programmed death 1 (PD-1) antibodies. *JAMA Neurol*. (2017) 74:1216–22. doi: 10.1001/jamaneurol.2017.1912
11. Sepúlveda M, Martinez-Hernandez E, Gaba L, Victoria I, Sola-Valls N, Falgàs N, et al. Motor polyradiculopathy during pembrolizumab treatment of metastatic melanoma. *Muscle Nerve*. (2017) 56:E162–7. doi: 10.1002/mus.25672
12. Yost MD, Chou CZ, Botha H, Block MS, Liewluck T. Facial diplegia after pembrolizumab treatment. *Muscle Nerve*. (2017) 56:E20–1. doi: 10.1002/mus.25663
13. Fellner A, Makranz C, Lotem M, Bokstein F, Taliansky A, Rosenberg S, et al. Neurologic complications of immune checkpoint inhibitors. *J Neuro-Oncol*. (2018) 137:601–9. doi: 10.1007/s11060-018-2752-5
14. Manam R, Martin JL, Gross JA, Chaudhary D, Chowdhary S, Espinosa PS, et al. Case reports of pembrolizumab-induced acute inflammatory demyelinating polyneuropathy. *Cureus*. (2018) 10:e3371—1. doi: 10.7759/cureus.3371
15. Ong S, Chapman J, Young G, Mansy T. Guillain–Barré-like syndrome during pembrolizumab treatment. *Muscle Nerve*. (2018) 58:E8–10. doi: 10.1002/mus.26101
16. Dubey D, David WS, Amato AA, Reynolds KL, Clement NF, Chute DF, et al. Varied phenotypes and management of immune checkpoint inhibitor-associated neuropathies. *Neurology*. (2019) 93e1093–103. doi: 10.1212/WNL.0000000000008091
17. Muralikrishnan S, Ronan LK, Coker S, Rauschkolb PK, Shirai K. Treatment considerations for patients with unresectable metastatic melanoma who develop pembrolizumab-induced guillain-barré toxicity: a case report. *Case Rep Oncol*. (2020) 13:43–8. doi: 10.1159/000504930
18. Vogrig A, Muàiz-Castrillo S, Joubert B, Picard G, Rogemond V, Skowron F, et al. Cranial nerve disorders associated with immune checkpoint inhibitors. *Neurology*. (2021) 96:e866–75. doi: 10.1212/WNL.0000000000011340
19. Berzero G, Picca A, Psimaras D. Neurological complications of chimeric antigen receptor T cells and immune-checkpoint inhibitors: ongoing challenges in daily practice. *Curr Opin Oncol*. (2020) 32:603–12. doi: 10.1097/CCO.0000000000000681
20. Sechi E, Markovic SN, McKeon A, Dubey D, Liewluck T, Lennon VA, et al. Neurological autoimmunity and immune checkpoint inhibitors: autoantibody profiles and outcomes. *Neurology*. (2020) 95:10632. doi: 10.1212/WNL.0000000000010632

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frontiers in Neurology

CASE REPORT
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Case Report: Mitochondrial Encephalomyopathy Presents as Epilepsy, Ataxia, and Dystonia With a Rare Mutation in *MT-TW*

Shuang Wang, Jing Miao and Jiachun Feng**

Department of Neurology, The First Hospital of Jilin University, Changchun, China

Mitochondrial diseases are a group of common inherited disorders caused by mutations in nuclear DNA or mitochondrial DNA (mtDNA); the clinical phenotype of diseases caused by mutant mtDNA is challenging owing to heteroplasmy of mtDNA and may delay diagnosis and treatment. Herein, we report the case of an adult male who slowly developed epilepsy, ataxia, dystonia, impaired cognition, and hearing impairment over 14 years in the absence of clinical myopathy. His lactate level was normal. Brain computed tomography showed calcifications of the bilateral basal ganglia, thalamus, and cerebellar dentate nuclei. Magnetic resonance imaging revealed multiple lesions in the bilateral internal capsule and periventricular areas, which were hypointense on T1-weighted images and hyperintense on T2-weighted images. The first blood genetic test result was negative. Two years later, a muscle biopsy was performed. Succinate dehydrogenase (SDH) staining showed several ragged blue fibers and atypical strongly SDH-reactive vessels. Cytochrome C oxidase (COX) staining revealed abundant COX-deficient fibers. mtDNA testing of blood and muscle revealed a rare m.5549G>A mutation in the *MT-TW* gene. It was heteroplasmic, with 5.4% mutant mtDNA in the blood and 61.5% in the muscle. The patient was diagnosed with mitochondrial encephalomyopathy and treated with levetiracetam instead of valproate to reduce possible mitochondrial toxicity. After receiving anti-epileptic drugs and mitochondrial supplements, the patient remained clinically stable. For mitochondrial disease, when mutant mtDNA is not detected in blood, muscle biopsy should be performed in routine analysis, and it should be genetically tested, even if there are no manifestations of myopathy.

Keywords: mitochondrial encephalomyopathy, *MT-TW*, epilepsy, ataxia, dystonia

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Costanza Lamperti, Fondazione IRCCS Istituto Neurologio Carlo Besta, Italy

***Correspondence:**
Jiachun Feng
fengjc@jlu.edu.cn
Jing Miao
jdyymiaojing@jlu.edu.cn

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INTRODUCTION

Mitochondrial diseases are caused by mutations in nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) and are characterized by dysfunction of oxidative phosphorylation (OXPHOS) and energy metabolism. Autosomal, X-linked, and maternal inheritance are all patterns of inheritance seen with mitochondrial diseases. It has been estimated that the prevalence of childhood-onset (<16 years) mitochondrial disease is 5–15 cases per 100,000 people, and in adults, the prevalence of causative mtDNA and nDNA mutations is estimated at 2.9 and 9.6 per 100,000 individuals, respectively (1, 2). The onset of mitochondrial diseases has a bimodal distribution, with one peak

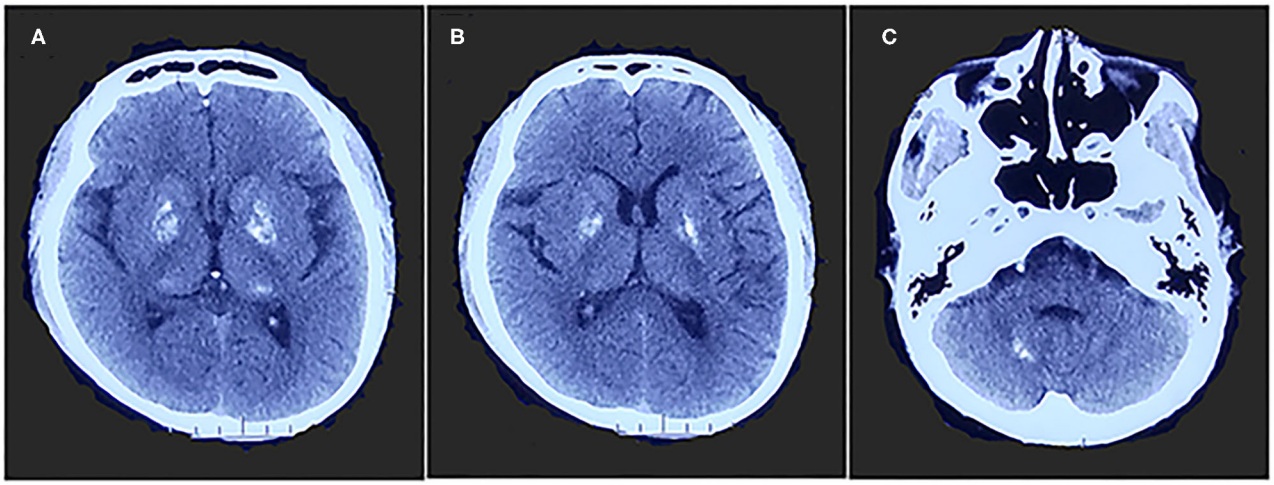

FIGURE 1 | Brain computed tomography shows calcifications of bilateral basal ganglia, thalamus **(A,B)**, and cerebellar dentate nuclei **(C)**.

before 3 years of age and the other at the end of adolescence to 40 years of age (2).

Mitochondrial diseases can involve any organ but especially affect organs that depend on aerobic metabolism, and these present with a range of symptoms. These diseases are clinically heterogeneous and characterized by epilepsy, progressive muscle weakness, dementia, ataxia, peripheral neuropathy, optic atrophy, hearing loss, stroke-like episodes, and diabetes mellitus. Some of the clinical features can be grouped into specific syndromes, such as Leigh syndrome, Kearns–Sayre syndrome, myoclonic epilepsy myopathy sensory ataxia, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes syndrome (MELAS), and myoclonic epilepsy with ragged red fibers (RRFs) syndrome. Common neuroimaging findings include white- or gray-matter lesions, atrophy, optic atrophy, stroke-like lesions, calcifications, and ischemic stroke (3). The diagnosis is complicated by variations in clinical phenotype and genotype. Currently, considerable advances in genetic testing technologies have been made, and most mutations in mtDNA can be detected in blood or urinary sediment. However, because mutant mtDNA heteroplasmy can vary across tissues (2), histochemical and biochemical analyses of tissue biopsies are essential for patients who have not been diagnosed by genetic testing of blood or urine samples.

Here, we report a case of adolescent-onset and slowly progressive mitochondrial encephalomyopathy in the absence of clinical myopathy caused by a rare m.5549G>A mutation in the *MT-TW* gene, which was diagnosed late due to the first negative blood mtDNA test finding that subsequently led to treatment delay.

CASE REPORT

A 26-year-old right-handed man presented with epilepsy, progressive worsening of gait imbalance, and involuntary movement for approximately 14 years. He had non-consanguineous parents and no relevant family history of neurological disease. When he was 12 years old, he experienced a tonic–clonic seizure. At that time, electroencephalography (EEG) showed extensive slow waves, especially in the posterior part of the brain. Brain computed tomography (CT) showed calcifications of the bilateral basal ganglia, thalamus, and cerebellar dentate nuclei (**Figure 1**). Magnetic resonance imaging (MRI) revealed hyperintense on T1-weighted images and hypointense or hyperintense on T2-weighted images in the bilateral basal ganglia, thalamus, and cerebellar dentate nuclei, which were coincident with areas showing calcifications in the CT images. The other lesions were slightly hypointense or isointense on T1-weighted images, with T2 hyperintense in the bilateral internal capsule and periventricular areas (**Figure 2A**). There were four episodes in the following 6 years. At the age of 18 years, he developed frequent absence seizures, and EEG showed generalized, symmetric, 3- to 3.5-Hz spike-and-wave discharge. The frequency of attacks was variable, but he did not receive any treatment. One year later, he developed gait instability and walked with a wide-based gait. MRI showed mild brain atrophy, but the lesions did not change significantly compared to those in the previous MRI (**Figure 2B**). His ataxia gradually worsened, and his parents found that he had developed impaired cognition. At the age of 22 years, his EEG showed generalized, low-to-medium amplitude, 3- to 4-Hz spike-and-wave discharge, and lesions in the MRI were unchanged (**Figure 2C**). He underwent blood genetic testing for the ataxia panel, which was negative, and he was treated with 500 mg of valproate twice daily. His clinical seizures were effectively controlled, but the ataxia and cognitive function did not improve.

At age 24 years, he presented with involuntary movements of his hands and mouth. The involuntary movement of the left hand was more severe than the right hand and manifested as a backward swinging motion, which was more obvious when

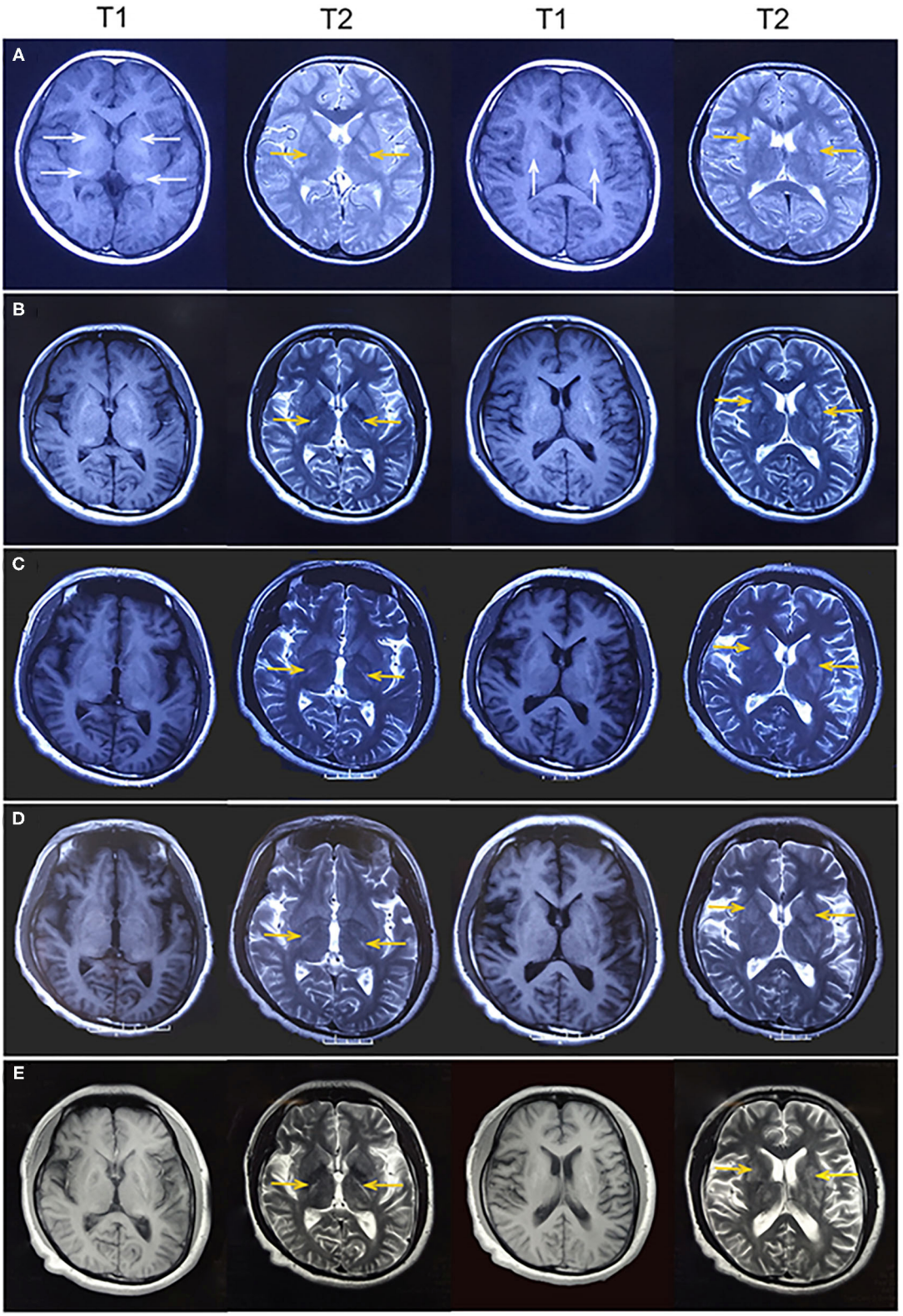

FIGURE 2 | (A) At age 12 years, magnetic resonance imaging (MRI) revealed hyperintense on T1-weighted images and hypointense or hyperintense on T2-weighted images in the bilateral basal ganglia and thalamus (white arrowhead), which were coincident with areas showing calcifications on computed tomography. Some lesions were slightly hypointense or isointense on T1-weighted images, with T2 hyperintense in the bilateral internal capsule and periventricular areas (yellow

(Continued)

FIGURE 2 | arrowhead). **(B)** At age 19 years, MRI showed mild brain atrophy, but the lesions of bilateral internal capsule and periventricular areas (yellow arrowhead) did not change significantly compared to those in the previous MRI. **(C–E)** At age 22, 24, and 26 years, MRI showed no change in the lesions of bilateral internal capsule and periventricular areas (yellow arrowhead). Brain atrophy showed no obvious change.

he was holding objects. In addition, the patient suffered from mild hearing loss. Laboratory findings for blood electrolytes, parathyroid hormone, copper, ceruloplasmin, and lactate levels were normal. An audiogram showed bilateral sensorineural hearing loss. Neurophysiological studies indicated mild sensory axonal damage. Echocardiography and electrocardiography findings were normal. MRI showed no change in the lesions (**Figure 2D**). Next-generation sequencing (NGS) indicated no definite pathogenic mutations in nDNA or mtDNA of blood. In the following 2 years, the patient's involuntary movement gradually worsened. At age 26 years, he visited our clinic with the abovementioned symptom (**Figure 3**). The patient denied weakness and bladder or bowel dysfunction. Neurological examination revealed dysarthria and no nystagmus. His limb strength was normal. Deep tendon reflexes were 1+. Bilateral pyramidal signs were positive, and no sensory disturbances were detected. A mental examination revealed an orientation obstacle. The Montreal Cognitive Assessment score was 24 and the Mini-Mental State Examination score was 26. Routine blood tests and laboratory examinations for creatine kinase, phytanic acid, amino acids, acylcarnitine, and lactate levels revealed normal findings. Urine test results for organic acids were also normal. Serologic tests for syphilis and human immunodeficiency virus showed negative results. There were no acanthocytes in the peripheral blood smear. The lesions on MRI were the same as before (**Figure 2E**). Magnetic resonance spectroscopy (MRS) revealed no lactate peaks.

As the patient had adolescent onset of symptoms and slowly developed epilepsy, ataxia, dystonia, impaired cognition, and hearing impairment along with the characteristic findings of symmetrical basal ganglia calcifications, leukoencephalopathy, and cerebral atrophy, mitochondrial disorder was suspected despite negative mtDNA blood test findings. Given that mutant mtDNA heteroplasmy can vary across tissues and defects in mtDNA typically present in post-mitotic tissues (2), we performed a biceps brachii biopsy after obtaining written consent from the patient. Hematoxylin and eosin staining showed no obvious abnormalities. Typical RRFs were not observed in modified Gomori trichrome staining. Succinate dehydrogenase (SDH) staining revealed ragged blue fibers (RBFs) and atypical strongly SDH-reactive vessels (SSVs). Cytochrome C oxidase (COX) staining revealed abundant COX-deficient fibers. COX-SDH staining showed only occasional COX-negative and SDH-positive fibers in blue (**Figure 4**). mtDNA testing of blood and muscle biopsy sample was performed, which detected a known m.5549G>A mutation in the *MT-TW* gene (4). It was heteroplasmic, with 5.4% mutant mtDNA in the blood and 61.5% in the muscle. The patient was diagnosed with mitochondrial encephalomyopathy. I_2, II_4, and II_7 agreed to carry out genetic testing of blood, but no mutations were detected in their blood.

The patient was previously treated with valproate. Considering its mitochondrial toxicity, we replaced valproate with levetiracetam. Mitochondrial supplements such as coenzyme Q10 (600 mg/day) and vitamins B2 (100 mg/day), B3 (250 mg/day), C (600 mg/day), and E (300 mg/day) were also administered. The patient was anxious before, but after the diagnosis of mitochondrial encephalomyopathy, he actively cooperated with the treatment and performed rehabilitation exercises. At the 1-year follow-up, he showed improvement in dystonia and ataxia, which remained clinically stable. As the symptoms stabilized, the increase in confidence of the patient was also conducive to treatment.

DISCUSSION

This case showed adolescent onset of symptoms and slow development of epilepsy, cerebellar ataxia, dystonia, impaired cognition, and hearing loss. He had characteristic imaging findings, including intracerebral calcification, white matter lesions, and brain atrophy. In muscle biopsy, RBFs, SSVs, and COX-deficient fibers were detected. In addition, a rare m.5549G>A heteroplasmic mutation in *MT-TW* was detected in the blood and muscle. All these manifestations were in line with the characteristics of mitochondrial diseases, and the patient was finally diagnosed with mitochondrial encephalomyopathy.

Our patient had abundant COX-deficient fibers in COX-stained samples. This is consistent with the characteristics of mutations in *MT-TW*. The *MT-TW* gene encodes the mitochondrial tRNA for tryptophan (tRNATrp). More than 10 mutations in *MT-TW* have been reported to date (5). Mutations in *MT-TW* may interfere with the structure and stability of tRNATrp, which can decrease the rate of mitochondrial protein synthesis and result in OXPHOS deficiency. Profound complex IV defects are a common feature of most tRNATrp mutations, which may be related to the higher percentage of tryptophan in the COI and COIII subunits (6, 7). Mutations in *MT-TW* can give rise to MELAS, neurogastrointestinal syndrome, Leigh syndrome, and mitochondrial myopathy (8–10).

The m.5549 G>A mutation in *MT-TW* is rarely found in mitochondrial diseases. In 1995, a patient reported to have the m.5549G>A mutation presented with progressive dementia, chorea, cerebellar ataxia, deafness, and peripheral neuropathy in the absence of clinical myopathy (4). That patient's symptoms began at the age of 40 years, developed rapidly and seriously, and led to death at the age of 53 years. His plasma lactate concentration increased after exercise, and CT showed severe atrophy of the whole brain with low-density lesions in the cerebral white matter. Muscle biopsy specimens showed COX-negative fibers, RRFs, and reduced complex I activity on polarography. Post-mortem examination showed heteroplasmic

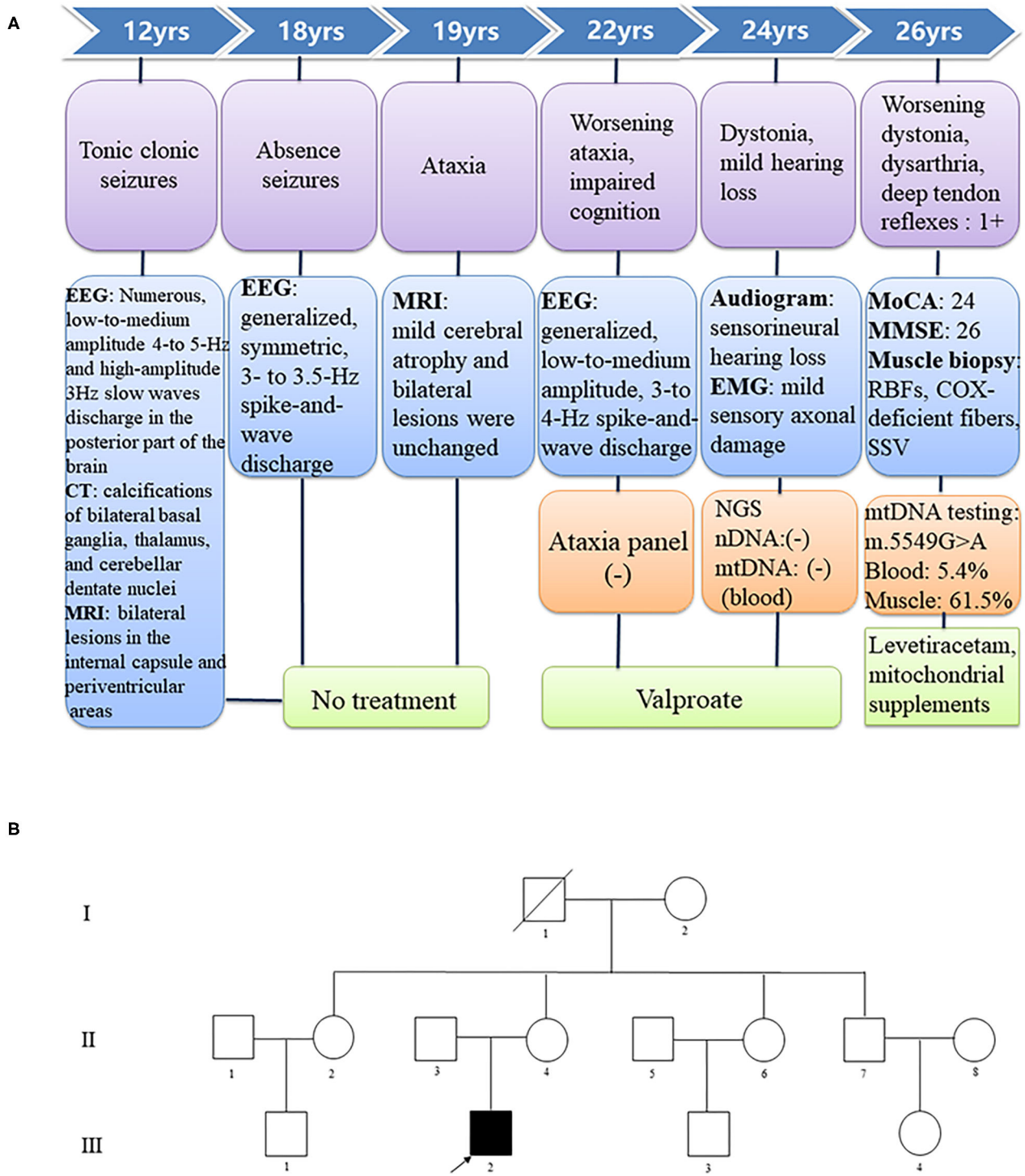

FIGURE 3 | (A) Chronology of the major clinical features. EEG, electroencephalography; CT, computed tomography; MRI, magnetic resonance imaging; EMG, electromyography; MoCA, Montreal Cognitive Assessment; MMSE, Mini-Mental State Examination; RBFs, ragged blue fibers; SSV, strongly succinate dehydrogenase-reactive vessel; COX, cytochrome C oxidase; NGS, next-generation sequencing; nDNA, nuclear DNA; mtDNA, mitochondrial DNA. **(B)** Pedigree of the family showing clinically and genetically affected members (black squares).

mutant mtDNA distributed in all tissues, ranging from 40% mutant mtDNA in blood to 93% in cardiac muscle. The proportions in the muscle and cerebral cortex were 92 and 87%, respectively. Compared with that patient, our patient differed in terms that he had adolescent onset, slow development of disease, and normal lactate levels. In his 14-year medical history, there

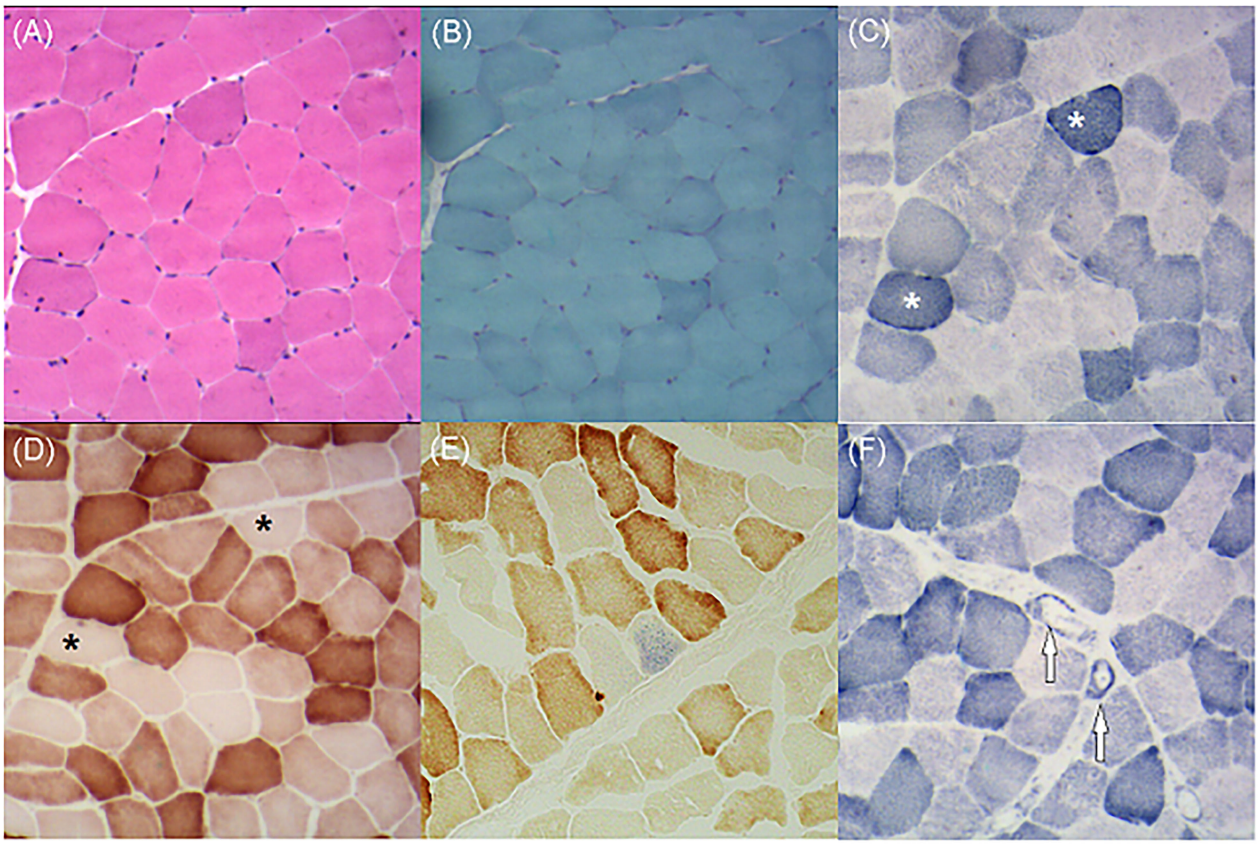

FIGURE 4 | (A) Hematoxylin and eosin staining demonstrates no obvious abnormalities. **(B)** Modified Gomori trichrome staining shows no ragged red fibers. **(C)** Succinate dehydrogenase (SDH) staining shows several ragged blue fibers (asterisks). **(D)** Cytochrome C oxidase (COX) staining shows plenty of COX-deficient fibers (asterisks). **(E)** COX-SDH staining shows occasional COX-negative and SDH-positive fibers in blue. **(F)** SDH staining shows atypical strongly SDH-reactive vessels (arrows).

was mild cerebral atrophy and symmetrical calcification. Bilateral lesions in the bilateral internal capsule and periventricular areas remained stable, providing additional imaging features for mitochondrial diseases. Muscle biopsy did not show RRFs, but RBFs, SSVs, and COX-deficient fibers were detected. In addition, the proportions of heteroplasmic mutations in the blood and muscle were 5.4 and 61.5%, respectively. The two cases have similarities in terms of clinical manifestations, but the age of onset, disease severity, and prognosis were obviously different. The difference between the two cases may be related to the proportion of mtDNA mutations in the affected tissue and the fact that the susceptibility of tissues to specific defects may change with the age of the patient (2). Both cases had a high percentage of mtDNA mutations in muscle in the absence of clinical myopathy and mainly presented with encephalopathy. These may be characteristics of the m.5549 G>A mutation in *MT-TW*. In our case, an interesting phenomenon is that the patient was clinically progressing but the brain MRI lesions seem to be stable. The brain MRI lesions existed 14 years ago, but he did not show ataxia or dystonia at that time. We speculate that with the development of the disease, the increase in the proportion of mutant mtDNA, the application of mitochondrial toxic drugs, and the long-term mental stress of the patient gradually revealed the patient's symptoms. In addition, MRS revealed no lactate peaks in our patient, because MRS is more sensitive to detect the lactate peak in the acute phase of mitochondrial disease. In patients with chronic MRI lesions, MRS may not detect the lactate peak (11).

The key features of our patient were cerebellar ataxia and dystonia. Their causes can be divided into acquired and genetic etiologies. In general, acquired causes can be ruled out based on laboratory tests or neuroimaging. Genetic testing is a valuable tool for determining the genetic etiology. When considering a certain disease, we can perform a relevant panel test. If the panel is negative or we are not sure about the diagnosis, NGS can be considered. For mitochondrial disease, the primary choice of tissue for genetic testing is blood, as harmful mitochondrial tRNA mutations may be actively eliminated in rapidly dividing cells (12). When mutant mtDNA is not detected in the blood, muscle biopsy should be performed in routine analysis, and these samples should be genetically tested, even if there are no manifestations of myopathy (13). Our patient had no mtDNA

mutation detected in the blood at the first mtDNA testing, whereas a mutation was detected 2 years later, but the proportion of mutations was only 5.4%. An increase in the proportion of mtDNA mutations in tissues may be attributed to disease progression. Since his first blood test was negative and a tissue biopsy was not performed, the diagnosis and treatment of the disease was delayed.

Currently, treatments for mitochondrial diseases are intended to slow down progression and alleviate symptoms. No Food and Drug Administration-approved drugs are presently available for the treatment of mitochondrial diseases (14). In addition to dietary regulation and mitochondrial supplementation, reducing the use of drugs with mitochondrial toxicity can delay disease progression. Valproate, carbamazepine, phenytoin, and phenobarbital are anti-epileptic drugs with mitochondrial toxicity (15). Valproate can isolate coenzyme-A and cytochrome-aa3, inhibit key enzymes of β-oxidation, and cause damage to the inner mitochondrial membrane and secondary carnitine deficiency. In addition, it can result in complex I and complex IV dysfunction and decreased ATP production (16). In this case, the patient was treated with valproate for 4 years before diagnosis, and we replaced valproate with levetiracetam. For mitochondrial diseases, gene therapy is still a popular research topic, and stem cell-derived mitochondrial transplantation has been shown to play a key role in metabolic rescue, which provides promise for mitochondrial encephalomyopathy (17). To date, there are a total of 49 registered clinical trials of new experimental drugs for the treatment of mitochondrial diseases, and 10 are Phase III trials. However, as of 2019, only one has been completed and the results have not been reported (14), and the treatment of mitochondrial diseases still faces many challenges.

CONCLUSIONS

This case demonstrates a rare mutation in *MT-TW* associated with epilepsy, ataxia, and dystonia in the absence of clinical myopathy, which was diagnosed late due to the first negative blood mtDNA test finding. For mitochondrial diseases, when blood mtDNA test result is negative, muscle biopsy should be performed in routine analysis, even if there are no manifestations of myopathy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JF and JM contributed to the conception and design of the manuscript. SW wrote the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Gorman GS, Schaefer AM, Ng Y, Gomez N, Blakely EL, Alston CL, et al. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Ann Neurol.* (2015) 77:753–9. doi: 10.1002/ana.24362
2. Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, et al. Mitochondrial diseases. *Nat Rev Dis Primers.* (2016) 2:16080. doi: 10.1038/nrdp.2016.80
3. Finsterer J. Cerebral imaging in adult mitochondrial disorders. *J Neurol Sci.* (2019) 404:29–35. doi: 10.1016/j.jns.2019.07.013
4. Nelson I, Hanna MG, Alsanjari N, Scaravilli F, Morgan-Hughes JA, Harding AE. A new mitochondrial DNA mutation associated with progressive dementia and chorea: a clinical, pathological, and molecular genetic study. *Ann Neurol.* (1995) 37:400–3. doi: 10.1002/ana.410370317
5. Cardaioli E, Mignarri A, Cantisani TA, Malandrini A, Nesti C, Rubegni A, et al. Myoclonus epilepsy, retinitis pigmentosa, leukoencephalopathy and cerebral calcifications associated with a novel m.5513G>A mutation in the MT-TW gene. *Biochem Biophys Res Commun.* (2018) 500:158–62. doi: 10.1016/j.bbrc.2018.04.009
6. Silvestri G, Mongini T, Odoardi F, Modoni A, deRosa G, Doriguzzi C, et al. A new mtDNA mutation associated with a progressive encephalopathy and cytochrome c oxidase deficiency. *Neurology.* (2000) 54:1693–6. doi: 10.1212/WNL.54.8.1693
7. Smits P, Mattijssen S, Morava E, van den Brand M, van den Brandt F, Wijburg F, et al. Functional consequences of mitochondrial tRNA Trp and tRNA Arg mutations causing combined OXPHOS defects. *Eur J Hum Genet.* (2010) 18:324–9. doi: 10.1038/ejhg.2009.169
8. Maniura-Weber K, Taylor RW, Johnson MA, Chrzanowska-Lightowlers Z, Morris AA, Charlton CP, et al. A novel point mutation in the mitochondrial tRNA(Trp) gene produces a neurogastrointestinal syndrome. *Eur J Hum Genet.* (2004) 12:509–12. doi: 10.1038/sj.ejhg.5201185
9. Blakely EL, Yarham JW, Alston CL, Craig K, Poulton J, Brierley C, et al. Pathogenic mitochondrial tRNA point mutations: nine novel mutations affirm their importance as a cause of mitochondrial disease. *Hum Mutat.* (2013) 34:1260–8. doi: 10.1002/humu.22358
10. Duff RM, Shearwood AM, Ermer J, Rossetti G, Gooding R, Richman TR, et al. A mutation in MT-TW causes a tRNA processing defect and reduced mitochondrial function in a family with Leigh syndrome. *Mitochondrion.* (2015) 25:113–9. doi: 10.1016/j.mito.2015.10.008
11. Mascalchi M, Montomoli M, Guerrini R. Neuroimaging in mitochondrial disorders. *Essays Biochem.* (2018) 62:409–21. doi: 10.1042/EBC20170109
12. Rahman S, Poulton J, Marchinton D, Suomalainen A. Decrease of 3243 A→ G mtDNA mutation from blood in MELASSyndrome: a longitudinal study. *Am J Hum Genet.* (2001) 68:238–40. doi: 10.1086/316930
13. Witters P, Saada A, Honzik T, Tesarova M, Kleinle S, Horvath R, et al. Revisiting mitochondrial diagnostic criteria in the new era of genomics. *Genet Med.* (2018) 20:444–51. doi: 10.1038/gim.2017.125
14. Weissig V. Drug development for the therapy of mitochondrial diseases. *Trends Mol Med.* (2020) 26:40–57. doi: 10.1016/j.molmed.2019.09.002
15. Finsterer J, Zarrouk Mahjoub S. Mitochondrial toxicity of antiepileptic drugs and their tolerability in mitochondrial disorders. *Expert Opin Drug Metab Toxicol.* (2012) 8:71–9. doi: 10.1517/17425255.2012.644535
16. Finsterer J, Zarrouk-Mahjoub S. Management of epilepsy in MERRF syndrome. *Seizure.* (2017) 50:166–70. doi: 10.1016/j.seizure.2017.06.010

17. Liu K, Zhou Z, Pan M, Zhang L. Stem cell-derived mitochondria transplantation: a promising therapy for mitochondrial encephalomyopathy. *CNS Neurosci Ther*. (2021) 27:733–42. doi: 10.1111/cns.13618

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frontiers in Neurology

CASE REPORT
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Acute Polyradiculomyelitis With Spinal Cord Gray Matter Lesions: A Report of Two Cases

*Charidimos Tsagkas†, Maria Janina Wendebourg†, Matthias Mehling, Johannes Lorscheider, Philippe Lyrer and Bernhard Friedrich Décard**

Neurology Clinic and Policlinic, Departments of Medicine, Clinical Research and Biomedical Engineering, University Hospital Basel and University of Basel, Basel, Switzerland

Objective: Inflammatory polyradiculomyelitis belongs to a rare group of immune-mediated diseases affecting both the central and peripheral nervous system. We aimed to describe an unusual presentation of acute polyradiculomyelitis with marked spinal cord lesions restricted to the gray matter.

Methods: Thorough examination of two case reports including clinical, MRI, serologic, electrophysiologic and CSF examinations as well as short-term follow-up.

Results: We present two adult patients with acute polyradiculomyelitis and unusual spinal cord lesions restricted to the gray matter on MRI. The clinical presentation, serologic, electrophysiologic and CSF features of the two patients varied, whereas both patients demonstrated severe, asymmetrical, predominantly distal, motor deficits of the lower extremities as well as bladder and bowel dysfunction. Both patients only partially responded to anti-inflammatory treatment. Severe motor impairment and bladder dysfunction persisted even months after symptom onset.

Conclusions: To our best of knowledge, these are the first reports of acute polyradiculomyelitis with distinct involvement of the lower thoracic spinal cord gray matter. Currently, it remains unclear whether gray matter lesions reflect a separate pathophysiologic mechanism or an exceedingly rare presentation of spinal cord involvement in acute polyradiculomyelitis.

Keywords: MRI, peripheral neuropathology, myelopathy, guillain-barre syndrome, clinical neurology, spinal cord gray matter lesions, AIDP

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Mamede De Carvalho, University of Lisbon, Portugal

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Sibel Karsidag, Maltepe University, Turkey
Catarina Falcão De Campos, Santa Maria Hospital, Portugal

***Correspondence:**
Bernhard Friedrich Décard
bernhard.decard@usb.ch
orcid.org/0000-0003-0118-8159

†These authors have contributed equally to this work

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INTRODUCTION

Inflammatory polyneuroradiculopathies are a group of immune-mediated diseases of the peripheral nerves and their spinal roots (1). Clinical manifestation usually involves distal onset and ascending sensory and/or motor deficits as well as autonomic dysfunction. Often, symptoms follow a preceding infection, most commonly with C. jejuni or respiratory viral pathogens (2). Symptom onset and progression may be acute or slow over a longer time span. Typical electrophysiological findings include slowing of nerve conduction velocity (NCV), conduction blocks and F-wave alterations, although early in the disease process, changes may not be detected (3). CSF analysis typically shows albuminocytological dissociation. The most effective therapy is intravenous immunoglobulin (IVIG) whereas rapid administration, especially in acute cases, is important.

Most common MRI findings include thickening of the cauda equina and spinal roots (4). Contrast enhancement of the conus, cauda equina and spinal roots is also compatible with the diagnosis (5), whereas it may disappear after treatment (6).

Rarely, myelitis accompanies inflammatory polyneuroradiculitis (7), resulting in polyradiculomyelitis. Viral or bacterial pathogens may be detected (8, 9).

However, to our knowledge, the combination of inflammatory polyneuroradiculitis and spinal cord (SC) lesions restricted to the gray matter (GM) has not been reported before. We present two cases of acute-onset inflammatory polyradiculomyelitis with GM lesions.

CASE REPORTS

First Case

A 56-year-old male with no previous neurologic history presented with severe lower back pain and reduced sensation as well as weakness of the distal right lower extremity. Within the next hours, these symptoms extended to the left leg and ascended proximally. Additionally, he complained about urinary retention and obstipation.

Clinically, he showed a predominantly right-sided, severe, flaccid, distal paraparesis and hypoesthesia involving dermatomes L4-S5 in all sensory modalities of both lower extremities. Spontaneous fasciculations were visible on the right M. quadriceps femoris. Stretch reflexes were absent in both lower extremities except for normal left quadriceps and adductor reflexes.

SC T2-weighted MRI of the lumbar spine revealed a hyperintense lesion extending from T12 to L1, including the conus medullaris with a butterfly-like shape on axial slices 1 day after admission. Post-contrast T1-weighted MRI showed enhancement of the cauda equina and subtle contrast enhancement of the SC lesion. A follow-up MRI 3 days later showed focal SC edema and persistence of subtle lesion contrast enhancement (**Figures 1A–E**). CSF analysis revealed an albuminocytological dissociation as well as an increased CSF/serum albumin quotient (**Table 1**). Serological analysis was positive for Campylobacter jejuni. Anti-ganglioside antibodies were unremarkable. NCV studies showed increased distal motor latencies. F-waves were either prolonged or absent (**Table 1**).

Hence, acute post-infectious polyradiculomyelitis was diagnosed. The patient was treated with IVIG 35g/d for 5 days. Under IVIG treatment, the patient improved significantly, particularly regarding proximal motor deficits, but was dismissed with a persisting severe, predominantly right-sided and distal, flaccid paraparesis and hypoesthesia as well as urinary retention and obstipation. The patient was transferred to a neuro-rehabilitation facility. After 3 months, his symptoms improved slightly further, but paraparesis and bladder dysfunction remained.

Abbreviations: NCV, nerve conduction velocity; IVIG, intravenous immunoglobulin; MOG, myelin oligodendrocyte glycoprotein; SC, spinal cord; GM, gray matter.

Second Case

A 62-year-old female with no previous neurologic history presented with acute pain and weakness in both lower extremities.

Clinically, she showed a mild, predominantly right-sided and distal paraparesis. The anal sphincter tonus was normal at that time point. Within a few hours, her symptoms deteriorated dramatically, and she developed a severe, flaccid, predominantly right-sided, distal paraparesis accompanied by urinary and stool incontinence. The patellar reflex was absent on the right side and brisk on the left side, whereas the achilles tendon reflexes were absent bilaterally.

SC T2-weighted MRI revealed a hyperintense lesion from T11 to L1, including the conus medullaris, with a butterfly-like shape on axial slices. Post-contrast T1-weighted MRI showed partial enhancement of the anterior parts of this lesion (**Figures 2A–D**). Brain MRI and CSF-analysis were unremarkable (**Table 1**). Two days after symptom onset, NCV studies showed absent F-waves of both lower extremities and a reduced compound muscle action potential of the M. extensor digitorum brevis. Electromyography demonstrated neither spontaneous nor voluntary activity of the right M. gastrocnemius, whereas examination of the left M. tibialis anterior showed signs of acute denervation. Somatosensory evoked potentials of tibial nerve were unremarkable on both sides. Motor evoked potentials of the right lower extremity were absent, whereas normal latencies were shown in the left lower extremity. Serologic analysis was unremarkable (**Table 1**). A poliovirus neutralization-test showed immunity.

Therefore, idiopathic acute polyradiculomyelitis was diagnosed. Assuming the myelitis was the leading cause for the symptoms, the patient was initially treated with methylprednisolone i.v., 500 mg daily for 5 days and consecutive oral prednisone. Under this regimen, the patient's symptoms improved slightly, but the paraparesis, bladder and bowel dysfunction were still severe. Therefore, 6 cycles of plasmapheresis were carried out. A further slight improvement of the motor symptoms became evident.

Thereafter, electrophysiological studies were repeated. Distal motor potentials of tibial and peroneal nerves were absent, whereas proximally (left N. femoralis), a compound muscle action potential amplitude reduction was shown. In contrast, sensory NCV studies of the lower extremities, e.g., the left sural and superficial peroneal nerve, were normal. Furthermore, the upper extremities showed normal NCV studies. Electromyography of the right vastus lateralis muscle showed spontaneous activity and increased recruitment frequency (>20/s). In addition, a follow-up SC MRI (22 days after the first MRI) showed new post-contrast enhancement of the cauda equina inT1-weighted MRI (**Figure 2D**). The SC T2-weighted hyperintensity remained stationary and showed subtle post-contrast enhancement in T1-weighted imaging.

After 3 months of neurorehabilitation, proximal pareses of the lower extremities were slightly improved but distal motor and bladder dysfunction persisted.

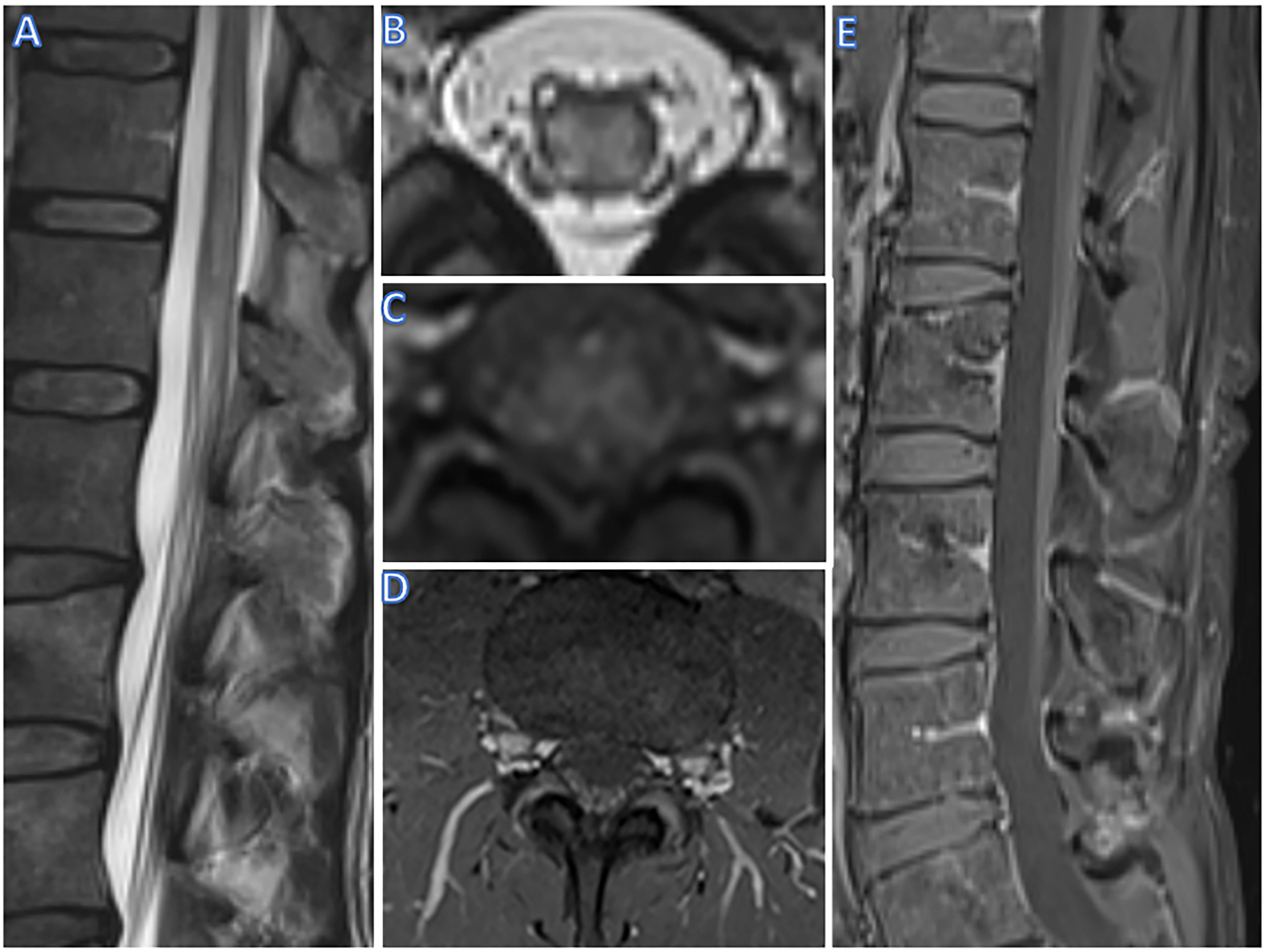

FIGURE 1 | R imaging of case 1 (male, 56 yrs). T2w hyperintense lesions and swelling of the conus **(A)** and selective hyperintensity and swelling of the gray matter on axial imaging **(B)**. On T1w, a diffuse contrast enhancement of the gray matter can be seen **(C)**, as well as contrast enhancement of spinal roots in axial **(D)** and sagittal imaging **(E)**.

DISCUSSION

We present two cases of acute polyradiculomyelitis with distinct involvement of the lower SCGM on MRI. Lesions restricted to the SCGM are not a common feature of acute polyradiculomyelitis. They have been described in acute SC infarction or chronic compressive myelopathy, leading to the typical "snake/owl's eyes" or "fried eggs" appearance on axial T2-weighted images (10, 11). In contrast to our patients, contrast enhancement in SC ischemia is absent in the acute phase. In addition, anterior horn lesions have been reported in some rare cases of West Nile virus-associated myelitis (12). However, due to a lack of a relevant travel history and missing typical accompanying symptoms such as high fever, neither patient was tested for West Nile virus. In addition, both patients formally fulfill the diagnostic criteria of the herpes simplex virus-associated Elsberg syndrome as described by Savoldi and colleagues (13) with the first patient meeting the criteria for a clinically definite and the second patient for a clinically probable diagnosis. However, in contrast to patients with Elsberg syndrome, our patients did not report signs of previous herpes simplex virus infection. Furthermore, Gorson and Ropper described two myelitis cases with polio-like anterior horn lesions mimicking a motor Guillain-Barre syndrome variant after an unspecific mild viral infection (14). Neither of our patients had reported symptoms of a previous viral infection. However, mild symptoms may simply not have been acknowledged. Moreover, polyradiculomyelitis has also been described in the context of both aquaporin-4 antibody positive neuromyelitis optica spectrum disorder and myelin oligodendrocyte glycoprotein (MOG) associated disorder (15–18). In particular, MOG-associated disorder frequently presents with longitudinally extensive lesions restricted in the SCGM of the thoracolumbar region, typically with absent contrast enhancement; in contrast, in patients with aquaporin-4 antibody positive myelitis, cervical and thoracic longitudinally extensive lesions with contrast enhancement are more frequent (19, 20). Indeed, both these differential diagnoses were considered in the clinical management of one of our patients (patient 2),

TABLE 1 | Overview of diagnostic measures applied to both patients.

	Patient A (male, 55 yrs)	**Patient B (female, 62 yrs)**
Serum analysis (pathological findings)	- Campylobacter jejuni titer: 1:96	- not measured
Further serum analysis (unremarkable findings)	- Anti-aquaporin-4 Ab: not measured - Anti-MOG Ab: not measured - Anti-ganglioside Ab negative - HIV screen negative - Lues screen negative - EBV, VZV, HSV I/II, CMV IgM negative - Poliomyelitis neutralization test: not measured	- Anti-aquaporin-4 Ab: negative - Anti-MOG Ab: negative - Anti-ganglioside Ab: negative - HIV screen: negative - Lues screen: negative - EBV, VZV, HSV I/II, CMV IgM: negative - Poliomyelitis neutralization test: proven immunity
CSF analysis	- Total protein 1,292 mg/l - Leukocytes 0 × 10^6/l - CSF/serum albumin quotient 20.1 × 10^{-3}	- Total protein 395 mg/l - Leukocytes 0 × 10^6/l - CSF/serum albumin quotient 5.6 × 10^{-3}
Neurography studies	- Day 1 after symptom onset: F-waves of lower extremities absent or elongated; distal motor latencies elongated - Follow-up examination: not conducted	- Day 2 after symptom onset: F-waves of lower extremities absent, CMAP of right EDB reduced - Day 20 after symptom onset: Motor nerves of lower extremities not measurable, sensory nerves of lower extremities intact
Myography studies	Not conducted	Signs of acute denervation in left gastrocnemius, right TA and gastrocnemius without any sign of voluntary or spontaneous activity
MR imaging (brain)	Unremarkable	Unremarkable
MR imaging (spinal cord)	- Day 1 after symptom onset: Gray matter myelopathy from Th11 to conus (L1); slight contrast enhancement of lumbar radices - Day 3 after symptom onset: Stationary gray matter myelopathy from Th11 to conus (L1); stationary slight contrast enhancement of lumbar radices	- At symptom onset: Gray matter myelopathy at Th11/12, no contrast enhancement at this point - Day 22 after symptom onset: Gray matter myelopathy at Th11/12; now contrast enhancement of lumbar radices visible

Ab, antibodies; CMAP, compound muscle action potential; CMV, cytomegalovirus; CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; EDB, M. extensor digitorum brevis; HSV I/II, herpes simplex virus type 1 & 2; MOG, myelin oligodendrocyte glycoprotein; TA, tibialis anterior; VZV, varicella-zoster virus.

which —however— tested negative for both anti-aquaporin 4 and anti-MOG antibodies. In addition, patient 1, who was not tested for any of those antibodies, did not demonstrate any additional typical signs and features of these disorders such as longitudinally extensive spinal cord lesions, brain or optic nerve involvement, recurrent and/or multifocal disease making these etiologies rather unlikely.

The underlying pathology for these MRI findings is unclear. Possibly, they reflect the aftermath of axonal injury occurring distally to the SCGM and leading to secondary Wallerian degeneration and axonal swelling reaching the neuron-somas. However, this hypothesis does not explain the presence of these lesions at symptom onset in our patients since axonal degeneration begins 36–44 h after nerve injury. Hence, a direct SCGM inflammatory involvement seems more plausible, which is also supported by the SCGM contrast enhancement in one of our patients. Despite the SC involvement in both patients, no pleocytosis was shown in CSF analysis, whereas one patient showed typical albuminocytological dissociation. Regarding the pathogenesis of our patients' disorder, bacterial and viral agents such as Campylobacter jejuni, Mycoplasma pneumoniae as well as the Zika and Dengue viruses have been described as triggers in both transverse myelitis and inflammatory polyneuroradiculitis (21). One of our patients tested positive for Campylobacter jejuni (the second patient was not tested), which may offer a pathogenetic explanation. Despite that, both patients had negative anti-ganglioside antibodies, which have been associated with these pathogens, although this is not unusual in all forms of polyneuroradiculitis (21).

With regard to the clinical features, both patients presented with atypical polyneuroradiculitis symptoms e.g., acute, distal, flaccid paralysis of the lower extremities, but also marked bowel and bladder symptoms. The latter most likely correspond to the SC involvement rather than the polyneuropathic component of the patients' disorder.

Notably, besides SC-associated symptoms, the two patients presented with different phenotypes; while the first patient showed sensorimotor deficits, the second patient presented a pure motor-fiber involvement. In patients with Elsberg syndrome, sensory nerve involvement may not be present. The pure motor fiber involvement may reflect the pronounced anterior horn lesions. Finally, despite early immunomodulatory

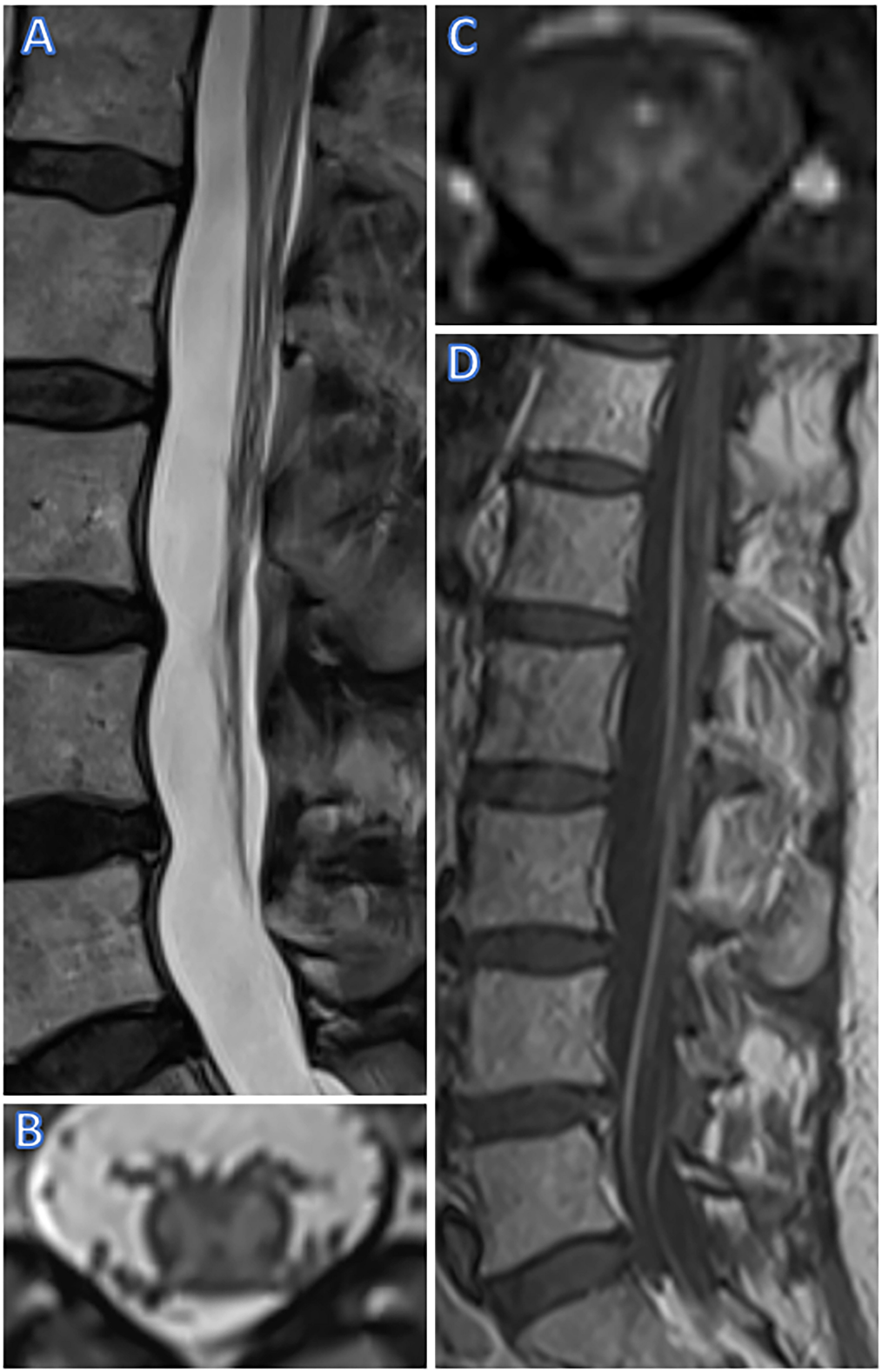

FIGURE 2 | MR Imaging of case 2 (female, 63 yrs). T2w sagittal imaging shows swelling and hyperintense lesions of the conus **(A)** while axial T2w imaging shows a butterfly-shaped lesion involving only the gray matter [at T11-12, **(B)**]. T1w imaging showed discreet contrast enhancement of the anterior horns on axial imaging **(C)**, and, on day 22 after onset, post-contrast T1w imaging also showed contrast of spinal cord roots **(D)**.

treatment, severe motor impairment and bladder dysfunction persisted even months after symptom onset. In the few reported cases of concomitant myelopolyradiculitis (22–24), long-term clinical outcomes varied, although pure motor variants seemed to be associated with a poorer prognosis (25).

To summarize, we report two cases of unusual SCGM lesions in patients with acute polyradiculomyelitis. Currently, it remains unclear whether this presentation reflects a separate pathophysiologic mechanism or an underappreciated manifestation of the inflammatory disease. Hence, future larger-scale studies should further investigate these findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

CT, MW, and BD drafted the manuscript and reviewed the data reported. All authors contributed to the clinical management of the reported patients and the revision and editing of the manuscript.

REFERENCES

1. Nobile-Orazio E. Chronic inflammatory demyelinating polyradiculoneuropathy and variants: where we are and where we should go. *J Peripher Nerv Syst JPNS.* (2014) 19:2–13. doi: 10.1111/jns5.12053
2. Hadden RD, Karch H, Hartung HP, Zielasek J, Weissbrich B, Schubert J, et al. Preceding infections, immune factors, and outcome in Guillain-Barré syndrome. *Neurology.* (2001) 56:758–65. doi: 10.1212/WNL.56.6.758
3. Berciano J, Orizaola P, Gallardo E, Pelayo-Negro AL, Sánchez-Juan P, Infante J, et al. Very early Guillain-Barré syndrome: a clinical-electrophysiological and ultrasonographic study. *Clin Neurophysiol Pract.* (2020) 5:1–9. doi: 10.1016/j.cnp.2019.11.003
4. Byun WM, Park WK, Park BH, Ahn SH, Hwang MS, Chang JC. Guillain-Barré syndrome: MR imaging findings of the spine in eight patients. *Radiology.* (1998) 208:137–41. doi: 10.1148/radiology.208.1.9646804
5. Alkan O, Yildirim T, Tokmak N, Tan M. Spinal MRI findings of Guillain-Barré syndrome. *J Radiol Case Rep.* (2009) 3:25–8. doi: 10.3941/jrcr.v3i3.153
6. Iwata F, Utsumi Y. MR imaging in Guillain-Barré syndrome. *Pediatr Radiol.* (1997) 27:36–8. doi: 10.1007/s002470050059
7. Guo F, Zhang Y-B. Clinical features and prognosis of patients with Guillain-Barré and acute transverse myelitis overlap syndrome. *Clin Neurol Neurosurg.* (2019) 181:127–32. doi: 10.1016/j.clineuro.2019.04.014
8. Poorthuis MHF, Battjes S, Dorigo-Zetsma JW, de Kruijk JR. Primary Epstein-Barr virus infection in immunocompetent patients with acute transverse myelitis and a combination of polyradiculitis and anterior horn syndrome as neurological manifestations. *BMJ Case Rep.* (2018) 2018:bcr2018225333. doi: 10.1136/bcr-2018-225333
9. Canpolat M, Kumandas S, Yikilmaz A, Gumus H, Koseoglu E, Poyrazoglu HG, et al. Transverse myelitis and acute motor sensory axonal neuropathy due to legionella pneumophila: a case report. *Pediatr Int Off J Jpn Pediatr Soc.* (2013) 55:778–82. doi: 10.1111/ped.12126
10. Al-Mefty O, Harkey LH, Middleton TH, Smith RR, Fox JL. Myelopathic cervical spondylotic lesions demonstrated by magnetic resonance imaging. *J Neurosurg.* (1988) 68:217–22. doi: 10.3171/jns.1988.68.2.0217
11. Novy J, Carruzzo A, Maeder P, Bogousslavsky J. Spinal cord ischemia: clinical and imaging patterns, pathogenesis, and outcomes in 27 patients. *Arch Neurol.* (2006) 63:1113–20. doi: 10.1001/archneur.63.8.1113
12. Kalita J, Vibhute A, Kumar M, Misra UK. Myelopathy in west Nile virus encephalitis: report of a case and review of literature. *J Spinal Cord Med.* (2020) 43:444–8. doi: 10.1080/10790268.2018.1507804
13. Savoldi F, Kaufmann TJ, Flanagan EP, Toledano M, Weinshenker BG. Elsberg syndrome: a rarely recognized cause of cauda equina syndrome and lower thoracic myelitis. *Neurol Neuroimmunol Neuroinflammation.* (2017) 4:e355. doi: 10.1212/NXI.0000000000000355
14. Gorson KC, Ropper AH. Nonpoliovirus poliomyelitis simulating Guillain-Barré syndrome. *Arch Neurol.* (2001) 58:1460. doi: 10.1001/archneur.58.9.1460
15. Rinaldi S, Davies A, Fehmi J, Beadnall HN, Wang J, Hardy TA, et al. Overlapping central and peripheral nervous system syndromes in MOG antibody–associated disorders. *Neurol Neuroimmunol Neuroinflammation.* (2020) 8:e924. doi: 10.1212/NXI.0000000000000924
16. Takai Y, Misu T, Nakashima I, Takahashi T, Itoyama Y, Fujihara K, et al. Two cases of lumbosacral myeloradiculitis with anti-aquaporin-4 antibody. *Neurology.* (2012) 79:1826–1828. doi: 10.1212/WNL.0b013e3182703ff7
17. Kim S, Park J, Kwon BS, Park J-W, Lee HJ, Choi J-H, et al. Radiculopathy in neuromyelitis optica. How does anti-AQP4 Ab involve PNS? *Mult Scler Relat Disord.* (2017) 18:77–81. doi: 10.1016/j.msard.2017.09.006
18. Toru S, Soejima I, Katayama Y, Saito K, Yokote H. A case of anti-AQP4 antibody-positive neuromyelitis optica spectrum disorder with MRI-proven lesions in lumbar nerve roots. *Mult Scler Relat Disord.* (2020) 46:102557. doi: 10.1016/j.msard.2020.102557
19. Dubey D, Pittock SJ, Krecke KN, Morris PP, Sechi E, Zalewski NL, et al. Clinical, radiologic, and prognostic features of myelitis associated with myelin oligodendrocyte glycoprotein autoantibody. *JAMA Neurol.* (2019) 76:301–309. doi: 10.1001/jamaneurol.2018.4053
20. Sato DK, Callegaro D, Lana-Peixoto MA, Waters PJ, Jorge FM de H, Takahashi T, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. *Neurology.* (2014) 82:474–81. doi: 10.1212/WNL.0000000000000101
21. Tripp A. Acute transverse myelitis and Guillain–Barre overlap syndrome following influenza infection. *CNS Spectr.* (2008) 13:744–7. doi: 10.1017/S1092852900013845
22. Sinha S, Prasad KN, Jain D, Pandey CM, Jha S, Pradhan S. Preceding infections and anti-ganglioside antibodies in patients with Guillain–Barré syndrome: a single centre prospective case-control study. *Clin Microbiol Infect.* (2007) 13:334–7. doi: 10.1111/j.1469-0691.2006.01636.x
23. Tolunay O, Çelik T, Çelik Ü, Kömür M, Tanyeli Z, Sönmezler A. Concurrency of Guillain-Barre syndrome and acute transverse myelitis: a

case report and review of literature. *Korean J Pediatr.* (2016) 59:S161–4. doi: 10.3345/kjp.2016.59.11.S161
24. Howell KB, Wanigasinghe J, Leventer RJ, Ryan MM. Concomitant transverse myelitis and acute motor axonal neuropathy in an adolescent. *Pediatr Neurol.* (2007) 37:378–81. doi: 10.1016/j.pediatrneurol.2007.05.020
25. Rodríguez Y, Rojas M, Pacheco Y, Acosta-Ampudia Y, Ramírez-Santana C, Monsalve DM, et al. Guillain–Barré syndrome, transverse myelitis and infectious diseases. *Cell Mol Immunol.* (2018) 15:547–62. doi: 10.1038/cmi.2017.142

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frontiers in Neurology

CASE REPORT
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Case Report: Plasma Biomarkers Reflect Immune Mechanisms of Guillain–Barré Syndrome

Chia-Lun Wu[1,2], *Chung-Hao Chao*[1], *Shun-Wen Lin*[1], *Yu-Yi Chien*[1,2], *Wen-Yi Huang*[1,2], *Wei-Chieh Weng*[1,2], *Feng-Chieh Su*[1] *and Yi-Chia Wei*[1,3]*

[1] Department of Neurology, Chang Gung Memorial Hospital, Keelung City, Taiwan, [2] School of Medicine, Chang Gung University, Taoyuan City, Taiwan, [3] Community Medicine Research Center, Chang Gung Memorial Hospital, Keelung City, Taiwan

This case series reported a group of patients with Guillain–Barré syndrome (GBS) and their plasma cytokine changes before and after immunotherapy. We aimed to understand GBS's pathogenesis and pathophysiology through observing the interval differences of the representative cytokines, which were the thymus and activation regulated chemokine (TARC) for T-cell chemotaxis, CD40 ligand (CD40L) for cosimulation of B and T cells, activated complement component C5/C5a, and brain-derived neurotrophic factor (BDNF) for survival and regenerative responses to nerve injuries. The fluorescence magnetic bead-based multiplexing immunoassay simultaneously quantified the five cytokines in a single sample. From June 2018 to December 2019, we enrolled five GBS patients who had completed before–after blood cytokine measurements. One patient was diagnosed with paraneoplastic GBS and excluded from the following cytokine analysis. The BDNF level decreased consistently in all the patients and made it a potential biomarker for the acute stage of GBS. Interval changes of the other four cytokines were relatively inconsistent and possibly related to interindividual differences in the immune response to GBS triggers, types of GBS variants, and classes of antiganglioside antibodies. In summary, utilizing the multiplexing immunoassay helps in understanding the complex immune mechanisms of GBS and the variation of immune responses in GBS subtypes; this method is feasible for identifying potential biomarkers of GBS.

Keywords: Guillain–Barré syndrome, cytokine, blood biomarker, Luminex, bead-based multiplexing immuno assay, immune mechanism, BDNF

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Hiu Yi Wong, Hong Kong University of Science and Technology, Hong Kong, SAR China

***Correspondence:**
Yi-Chia Wei
yichiawei@gmail.com

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INTRODUCTION

Immune Mechanism of Guillain–Barré Syndrome

Guillain–Barré syndrome (GBS) is an inflammatory disease of the peripheral nervous system induced by aberrant immune responses to preceding triggers. The pathogenesis of GBS is partly due to molecular mimicry of antecedent pathogens and subsequent provocation of generating cross-reactive antibodies that target different gangliosides in human peripheral nerves. Gangliosides are polymorphic sialic acid–containing glycosphingolipids that are widely distributed in the nervous system (1). Once the immune system responds to gangliosides as microbial mimics, immune-mediated neuropathies develop. Characteristic antiganglioside antibodies in peripheral blood mark several GBS

variants. In acute motor axonal neuropathy (AMAN), antibodies bind to GM1 and GD1a gangliosides in the pathogenesis of nerve injuries (2–5). In contrast, anti-GQ1b antibodies are associated with the Miller Fisher syndrome (6). In addition to disease correlations, these autoantibodies against axonal targets are indicators of GBS severity (7).

Biomarkers of GBS

Beyond antiganglioside antibodies that featured GBS, a growing number of molecules are potential biomarkers of GBS (8), including infection trigger-associated surface molecules (e.g., lipo-oligosaccharides of *Campylobacter jejuni*), active components of immune systems (e.g., FcγR/FcRL gene polymorphism, cytokines, complements, chemokines), brain-derived proteins (e.g., total protein, albumin), and neuronal composition (e.g., neurofilaments) (9–11). These biomarkers target different critical points of pathogenesis and neuronal damage in GBS.

Blood cytokines reflect elicitation of autoimmunity and disease severity in systemic autoimmune diseases, such as interleukin 6 (IL-6) and IL-10 in systemic lupus erythematosus (12). In immune-mediated neurological disorders, blood cytokines also emerge to be potential biomarkers, such as the B-cell–activating factor (BAFF) in myasthenia gravis with anti–acetylcholine receptor antibody (13, 14) and chronic inflammatory demyelinating polyneuropathy (CIDP) (15, 16). When comparing GBS patients with healthy controls, specific blood cytokines increase, including the tumor necrosis factor α (TNF-α), IL-1β, IL-6, IL-4, IL-17, and interferon γ (17). In this study, we hypothesized that blood cytokines of different parts of immune systems could reflect immune activation in GBS and patients' response to treatment. We followed a group of GBS patients during treatment and used a multiplex quantitative cytokine assay to measure their plasma cytokine changes before and after treatment. The selected cytokines represent the center of the neuroinflammation of GBS. A member of the TNF family, BAFF, appears for survival of antibody-producing B cells (18). The thymus and activation regulated chemokine (TARC), also known as CCL17, represent helper T cell 2 (T_H2)–induced T-cell chemotaxis (19). The CD40 ligand (CD40L), also known as CD154, expresses mainly on activated $CD4^+$ T cells, binds to the CD40 on B cells and antigen-presenting cells, and stands for cosimulation of B and T cells (20). C5 and C5a components (C5/C5a) are activated fragments of the complement system (21). Finally, the brain-derived neurotrophic factor (BDNF) measures the neuronal survival responses to GBS-related nerve injuries (22). By measuring these representative cytokines, we aimed to understand GBS pathogenesis and pathophysiology to identify potential biomarker(s).

MATERIALS AND METHODS

Patient Enrollment

The patients with GBS were enrolled from June 2018 to December 2019 in the Chang Gung Memorial Hospital, Keelung City, Taiwan. The enrolled patients understood and agreed to join the study and signed written informed consent before having the first (before-treatment) peripheral blood sampling, antiganglioside antibody detection, and cytokine measurement. We excluded those patients without complete before–after blood cytokine sampling. Besides, clinicians arranged cerebrospinal fluid (CSF) studies for biochemistry analyses based on their clinical judgment. This study was approved by the institutional review board of Chang Gung Medical Foundation, with approval number 201700701A3.

Ganglion Glycosphingolipid (Ganglioside) Antibody Detection

We performed ganglioside antibody detection on the EUROLINE platform manufactured by the EUROIMMUN (Lübeck, Germany). Samples were prepared by mixing 30 μL of plasma in 1.5 mL of 1:10 diluted sample buffer. Diluted samples were incubated with testing strips precoated with ganglioside antigens GM1, GM2, GM3, GD1a, GD1b, GT1b, and GQ1b. After incubation, the strips were washed to remove extra uncoated samples and then incubated with the enzyme conjugate, which was alkaline phosphate–labeled anti–human immunoglobulin G (IgG) and IgM (goat) to detect antiganglioside IgG and IgM in the sample, respectively. Another washing step removed the secondary antibodies. Next, the strips were incubated with the substrate, nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (BNT/BCIP). The strips were air-dried and evaluated by the EUROLINE semiquantitative software.

Quantification of the Cytokines by Fluorescent Bead–Based Multiplexing Immunoassay

The Luminex assay (Magnetic Luminex Assay: Human Premixed Multi-Analyte Kit; R&D Systems, Minneapolis, MN, USA) was a bead-based multiplexing immunoassay. Using fluorescent flow cytometry technique, the Luminex quantified multiple targets in one sample (23, 24). The plasma from GBS patients was mixed with the cytokine-specific capture antibodies coated on magnetic microparticles. In this study, the magnetic microparticle cocktail contained five kinds of precoated particles with capture antibodies against BDNF, C5/C5a, CD40L, TARC, and BAFF. The cytokine-capturing magnetic particles were mixed with the secondary detection antibody cocktail to form antibody–antigen–antibody complexes. Later, the embedded fluorophores bound to streptavidin–phycoerythrin conjugate and then excited by lasers. Finally, the Luminex analyzer followed the mechanism of flow cytometry to sort magnetic microparticle mixtures and quantified each cytokine independently. Each sample was repeated for three measurements.

RESULTS

Participants and Clinical Course

Among 10 patients who met the diagnostic criteria of GBS (25), five of them completed before and after treatment cytokine testing. Their clinical scenarios are listed below and summarized in **Table 1**.

TABLE 1 | Clinical and laboratory studies of the enrolled patients.

	Case 1	Case 2	Case 3	Case 4	Case 5
Basic information					
Age	28	16	49	71	57
Sex	Female	Female	Female	Male	Male
Medical history	CN III palsy	None	Type 2 DM	RA, HTN	NMO, SS
Clinical presentations					
Diagnosis	AIDP	AIDP	AIDP	MFS	AMSAN and myelitis
Onset	Subacute	Subacute	Acute	Acute	Acute
Symptoms	Dysarthria, dysphagia	Ataxic gait, limbs and facial numbness, and right CN VII palsy	Four limb weakness, ascending numbness, left CN VI and bil CN VII palsy, dysphagia, dysarthria, dysautonomia	Cerebellar ataxia, four-limb ascending numbness, bil CN III, IV, and VI palsy	Acute descending numbness below T5 level; subsequent ascending numbness
Prodrome	None	None	None	URI	None
Cancer association	None	None	None	Prostate cancer	None
Treatment	DFPP, steroid	IVIG, steroid	DFPP	DFPP, steroid	DFPP, AZA, steroid
Outcome	Good	Good	Partial	Good	Partial
Clinical studies					
NCS/EMG	Dem, M	Dem-Ax, M	F-wave absent	Ax, S-M	Ax, S-M
Spine MRI	n/a	Normal	Normal	n/a	T3-5 myelitis
Brain MRI	Normal	n/a	Normal	WMH	Pontine myelinolysis
CSF [protein (mg/dL)/WBCs (per μL)]	39.7/0	101.2/10	181.3/0	29.9/0	128.7/190 (Lym 83/Mo 16/Neu 1)
Paraprotein in CSF	None	None	None	IgA-lambda	None
Autoantibody					
Antiganglioside ab	GM1 IgM	GM2 IgM	GQ1b IgG	GQ1b IgG	GM1 IgM
Paraneoplastic ab	None	n/a	None	Yo	n/a
Other abs	None	None	None	None	AQP4, SSA
Plasma cytokine test					
From onset to first test	6 days	5 days	8 days	–	6 days
Days between tests	96 days	7 days	9 days	–	52 days
Treatment before the first blood test	None	None	None	–	Methylprednisolone 1,000 mg/day

NCS/EMG, nerve conduction study and electromyography; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; WBCs, white blood cells; CN, cranial nerve; DM, diabetes mellitus; RA, rheumatoid arthritis; HTN, hypertension; NMO, neuromyelitis optica; SS, Sjögren syndrome; AIDP, acute inflammatory demyelinating polyradiculoneuropathy; MFS, Miller Fisher syndrome; AMSAN, acute motor–sensory axonal neuropathy; Bil, bilateral; URI, upper respiratory tract infection; DFPP, double-filtration plasmapheresis; IVIG, intravenous immunoglobulin; AZA; azathioprine. Dem, demyelinating; Ax, axonal; M, motor; S-M, sensorimotor; n/a, not available; WMH, white matter hyperintensity; Lym, lymphocyte; Mo, monocyte; Neu, neutrophil; ab, antibody.

Case 1

A 28-year-old woman, a carrier of hepatitis B, presented with acute onset of dysphagia and dysarthria for 1 week. She came to our hospital, where the neurological examination found a decrease of bilateral gag reflex. The nerve conduction studies revealed generalized demyelinating polyneuropathy with conduction block, prolonged F-wave, and decreased amplitude. A CSF study showed white blood cells (WBCs) of 0/μL and total protein of 39.7 mg/dL. Antiganglioside antibody testing found GM1 IgM in her blood. Under the diagnosis of acute inflammatory demyelinating polyradiculoneuropathy (AIDP), she received one cycle (five sessions) of double-filtration plasmapheresis (DFPP) plus oral corticosteroid and recovered completely.

Case 2

A 16-year-old girl was admitted to the hospital for subacute-onset ataxic gait, four-limb and facial numbness, and right cranial nerve (CN) VII palsy. Nerve conduction studies showed generalized, mixed type with demyelinating predominant motor neuropathy. Albuminocytological dissociation of CSF study (WBCs 10/μL, total protein 101 mg/dL) and GM2 IgM antibodies in blood supported the diagnosis of AIDP. After intravenous immunoglobulin (IVIG) and steroid treatment, she had total recovery.

Case 3

A 50-year-old woman with type 2 diabetes mellitus presented to our hospital for weakness and ascending numbness over four limbs, left CN VI and bilateral CN VII palsy, dysphagia,

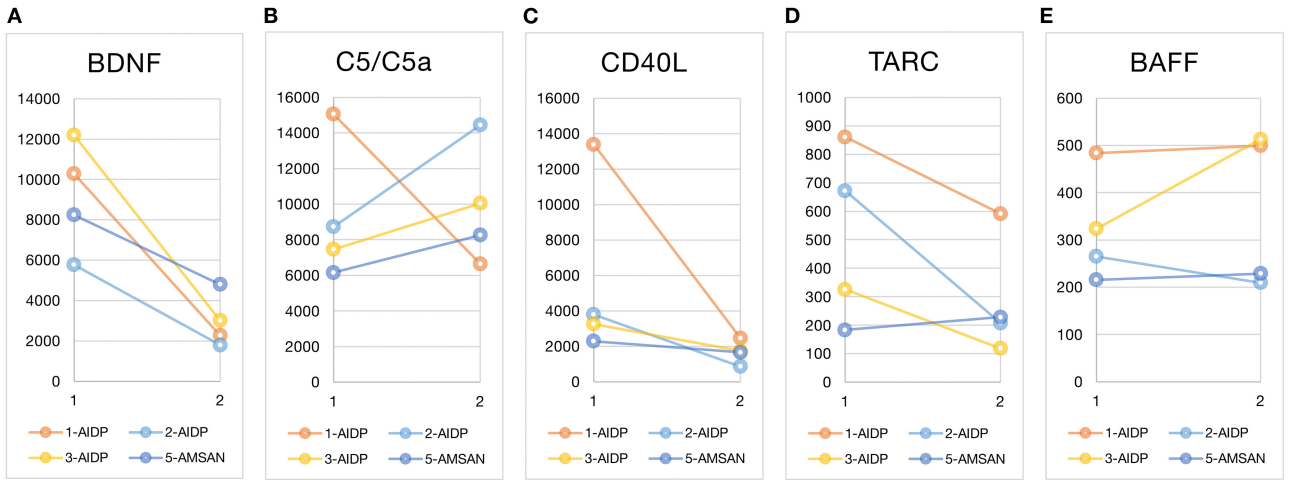

FIGURE 1 | Before and after treatment plasma cytokine changes. Luminex multiplexing assay measured the five cytokines at two time points: at GBS diagnosis (before treatment) and after immunotherapy. The line graphs marked the before–after cytokine changes of each patient (the x-axis time point 1 for before treatment and 2 for after treatment). The unit of cytokines was μg/mL. The case number and type of GBS variants are listed in the graphic legend. Case 4 was excluded from the cytokine comparisons because of the obvious paraneoplastic nature of GBS in this case and the heterogeneity from other nonparaneoplastic GBS. BDNF, brain-derived neurotrophic factor; C5/C5a, the activated complement component C5 and C5a; CD40L, CD40 ligand; TARC, thymus and activation regulated chemokine; BAFF, B-cell–activating factor; AIDP, acute inflammatory demyelinating polyradiculoneuropathy; AMSAN, acute motor-sensory axonal neuropathy.

dysarthria, and dysautonomia for 4 days. Brain and spine magnetic resonance imaging (MRI) showed no peculiar finding. F-wave was absent in nerve conduction studies. The sensory-evoked potential study suggested a generalized sensory conduction defect at peripheral levels. Blood antiganglioside antibody test was positive for anti-GQ1b antibody. After DFFP for AIDP, her recovery was partial, with sequelae of limb weakness and numbness.

Case 4

A 70-year-old man with a history of rheumatoid arthritis, hypertension, glaucoma, and traumatic subdural hemorrhage presented to our neurologic department for acute-onset cerebellar ataxia, four-limb ascending numbness, and palsies of bilateral CN III, IV, and VI. The results of the MRI of the brain were normal. Nerve conduction studies showed a pattern of chronic generalized axonal sensorimotor peripheral neuropathy. However, progressive eye movement limitations developed in the following 2 weeks. CSF study found WBCs of 0/μL and a total protein of 29.9 mg/dL. Anti-GQ1b antibody and anti-Yo antibodies were positive in his blood. Cancer surveillance found prostate cancer. Under the impression of Miller Fisher syndrome superimposed on paraneoplastic cerebellar degeneration, the patient received steroid pulse therapy and two courses of plasmapheresis. After that, his ataxia and CN palsies improved well.

Case 5

A 57-year-old man with a history of neuromyelitis optica (NMO) and Sjögren syndrome was admitted for recurrent myelitis presenting as acute descending numbness below T5 level for 1 week. However, subsequently ascending numbness from feet to thigh developed 1 month after the acute myelitis. A nerve conduction study revealed generalized axonal-type sensorimotor polyneuropathy. In addition to anti-aquaporin4 (AQP4) and anti-SSA antibodies, we also found GM1 IgM antibodies in his blood. He was diagnosed with acute motor–sensory axonal neuropathy (AMSAN) and recurrent myelitis of NMO. After treatment of DFFP, steroid, and azathioprine, his symptoms partially recovered, leaving him with numbness and weakness of the lower limbs.

All the enrolled patients were positive for antiganglioside antibodies, including two for GM1 IgM, one for GM2 IgM, and two for GQ1b IgG autoantibodies (**Table 1**). Specific antiganglioside antibodies are associated with certain GBS variants, such as the anti-GQ1b antibody's relationship to Miller Fisher variant and GBS with ophthalmoplegia (26), as our cases 3 and 4. Additional antibodies detected in the patients' blood included anti-Yo antibody in the patient with prostate cancer (case 4) and anti-AQP4 antibody and anti-Ro/SSA antibody in the patient with NMO and Sjögren syndrome (case 5) (**Table 1**).

Luminex Cytokine Quantification

Notably, case 4 was apparently a case of paraneoplastic GBS because of the newly diagnosed malignancy with high cancer activity and paraneoplastic anti-Yo antibody in his blood. Therefore, to avoid heterogeneity of studying group, case 4 was excluded from the following Luminex cytokine quantification.

Figure 1 shows the plasma cytokine levels before and after treatment of the four nonparaneoplastic GBS patients (cases 1, 2, 3, and 5). BDNF level decreased after treatment in all these patients. The before–after change could be up to fourfold in some patients (**Figure 1A**). The activated complement C5/C5a increased significantly in case 2 and increased slightly in the other two cases (cases 3 and 5) but decreased in case 1 (**Figure 1B**). The level of soluble form CD40L was initially high in case

1 and dropped after treatment, whereas the slope of CD40L decrease was less steep in cases 2 and 3 (**Figure 1C**). TARC concentration ranged from 100 to 900 μg/mL in our patients; TARC showed a downward trend at different levels in cases 1, 2, and 3 after treatment (**Figure 1D**). The plasma level of BAFF ranged between 300 and 500 μg/mL; testing sensitivity under low concentration circumstances limited the interpretation of BAFF's interval changes (**Figure 1E**).

The interval of the first symptom to the first blood cytokine test ranged between 5 and 8 days (median of 6 days). The days between the two cytokine tests ranged from 7 to 96 days (median, 30.5 days), depending on the patient's condition to reach clinical stabilization to have the after-treatment blood sampling. Three of the four patients were not exposed to immunotherapy before blood sampling. One patient (case 5) had started steroid pulse therapy (methylprednisolone 1,000 mg/day) before the first blood sampling (**Table 1**).

DISCUSSION

Summary

We reported four GBS patients and compared their plasma cytokine levels before and after immunotherapy. Using the Luminex multiplexing assay, we reduced the required amount of blood sample and measured multiple cytokines simultaneously. Two of the measured molecules, the BDNF (27) and the activated complement C5 (28, 29), have been deemed potential biomarkers or therapeutic targets for GBS. In contrast, the changes of plasma BAFF, CD40L, and TARC in GBS was reported for the first time.

The before–after change of BDNF was relatively consistent in our patients, and the potential of BDNF as an acute phase biomarker of GBS warranted large replication studies to confirm. Interindividual variations and interval changes of C5/C5a, CD40L, TARC, and BAFF level could be related to inconsistent disease phases at blood sampling, different triggers, immune responses, and interpersonal variations of responses to immunotherapy. Still, we could see a trend of early elevation of CD40L and TARC level and later elevation of C5/C5a level. Case 2 was the case that showed higher CD40L and TARC levels than other cases, which might be because the patient had previously encountered autoimmune neuropathy with CN III palsy and, for this episode, had a more robust cytokine response in the second-time confronting.

Complement Activation in GBS

Of notice, GBS has complicated immune mechanisms during disease progression, which involve infection-induced immune mimicking, antiganglioside antibody–mediated immune reaction, imbalanced T-cell activity, and macrophage infiltration. First, antiganglioside antibodies are considered the mediators of complement activation (30). The complements activated by the antiganglioside autoantibodies lead to the formation of membrane attack complex, disrupt expression of sodium channel, result in conduction block, and then exhibit the clinical signs of nerve damage (31). Some *in vitro* studies supported the concept that C3b and C5b-9 had harmful effects on peripheral nerves (8, 32). Complement-activated deposition of C3b on the outer surface of Schwann cells can lead to the initiation of vesiculation of myelin. Infiltration of activated macrophages and T cells follows the myelin break and subsequently induces axonal degeneration (33, 34). A serial observation found that complements kept aggregating around nerves where the blood–nerve barrier was broken and led to nerve injury during the first 4 weeks of GBS (28). We also observed a delayed elevation of complement active components C5/C5a. The relatively high level of C5/C5a did not appear at the initial stage but at a later stage in most of our patients (cases 2, 3, and 5). Therefore, the complement-mediated nerve injury did not quickly cease and might be the reason for persistent limb weakness or numbness.

T-Cell Immunity in GBS

Different groups of T cells participate in the pathogenesis of GBS. $CD4^+$ helper T cell dysregulation goes through the entire disease course of GBS. At the initial phase of GBS, T_H1 proinflammatory activity is upregulated. In the later stage, the upregulation of the T_H2 anti-inflammatory cytokine replaces the T_H1 cytokine activity (35). Together with the T_H1 cells, circulating T_H17 and T_H22 cells are also significantly increased in GBS patients, correlated with disease severity, and downregulated in response to IVIG treatment (36). Regulatory T (Treg) cell is another group of T cells that critically mediates the autoimmunity of GBS. Temporarily reducing of circulating Treg is related to the loss of its negative regulations on immune response in GBS (37, 38). Augmentation of Treg rescued nerve injuries in the experimental autoimmune neuritis (EAN) animal model (39). On the contrary, $CD8^+$ cytotoxic T cells increase in peripheral blood (40) and infiltrate endoneurium, especially in those patients with a subacute clinical course of GBS (28). To summarize, imbalanced T-cell function is crucial for the development of GBS. Antagonistic effects among the T_H1, T_H2, T_H17, T_H22, and Treg cells determine the development, progression, or recovery of GBS (41).

In our patients, plasma TARC and CD40L levels initially elevated and later dropped in some patients (cases 1, 2, and 3) but kept unchanged at a low level in the other one (case 5, **Figure 1**). Although the inconsistency might represent interindividual differences of T-cell activation, the type of GBS variant might matter. In a study of lymphocyte subset, the AIDP group showed significantly higher percentages of $CD4^+CD45RO^+$ memory T cells and lower percentage of $CD4^+CD45RA^+$ naive T cells than the healthy control; this ratio reversed after IVIG treatment. However, the AMAN variant did not possess this disparity to the healthy control or the before–after difference (42). The significant before–after changes of TARC and CD40L in our AIDP patients (cases 1, 2, and 3) might also reflect the T-cell involvement in AIDP type but not in other variants (case 5).

Costimulatory Molecules in GBS

Costimulatory molecules increase in number and enhance the cellular immune responses in several autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes mellitus, and multiple sclerosis (43); using monoclonal antibodies targeting costimulatory molecules is one of the developing treatments of autoimmune diseases (44). The CD40

and CD40L are a pair of costimulatory molecules between B cells, macrophage, dendritic cells, and activated T cells; upregulation of CD40 appears together with the increase of plasmacytoid dendritic cells in the acute phase of GBS patients (45). Also, in the animal model of GBS, CD40 is essential in creating EAN in mice (46); the dramatically increased expression of CD40 and CD40L marks the cooperation of B and T cells in the initiation of neuritis (47). Although enhanced expression of other costimulatory molecules has already been shown in GBS, such as the CD80 and CD86 (i.e., the B7-1 and B7-2 costimulatory molecules) (48) and the inducible T-cell costimulator (49), the CD40L was first shown in our report to be involved in pathogenesis and be a potential biomarker in the acute phase of GBS.

Chemokines in GBS

Trafficking inflammatory cells across the blood–nerve barrier is crucial in developing GBS; chemokines and chemokine receptors express in the endoneurium of peripheral nerves and circulate in the blood of GBS patients and EAN animal models (50). Previous studies of GBS and EAN have identified several chemokines and their receptors as pathogenic marks, including CCL2-CCR2 (51) and CXCL10-CXCR3 (52). Some others were considered treatment targets, although some succeed (such as CCR2) (53) and some failed (like CCR5) (54). Except the aforementioned chemokines, the CCR4 family is the other potential pathogenic target of GBS; positive staining of CCR4 was shown in the sural nerve biopsy of AIDP patients and was localized on invading T cells (55). The importance of CCR4 and its two ligands, TARC (CCL17) and CCL22, has also been noticed in central nervous system autoimmunity and studied in the experimental autoimmune encephalomyelitis murine model (56). To our knowledge, this is the first report that identified TARC (CCL17) as a potential biomarker of acute GBS, and the results warranted replication and animal model confirmation.

B-Cell Immunity in GBS

In previous studies, the B cells seem not at the center of GBS pathogenesis. The peripheral blood B-cell subset did not alter in GBS (42). However, increase in memory B-cell ratio in GBS patients with IgG antiganglioside antibodies suggested the antibody-initiated immune chain reaction (57). In our study, only case 3 had a before–after change of BAFF concentration, but not significant (**Figure 1**). Of notice, case 3 was positive for IgG antiganglioside antibodies, whereas the other cases were positive for IgM antiganglioside antibodies (**Table 1**). Although we could not confirm the differences of B-cell subsets between IgG- and IgM-related GBS, the slight increase of BAFF level in our patients might echo the importance of B-cell immunity in IgG-related GBS. Similarly, BAFF plays a key role in CIDP and determines if the patient responds to IVIG (15, 16).

In addition to that, antiganglioside antibodies are pathogenic in GBS (58, 59). Gangliosides are widely distributed on the outer leaflets of plasma membranes of various tissues but particularly abundant in neuronal cells. The sialic acids and negative charge of gangliosides make them form a protective shield to avoid autologous immunity and against pathogen attachment. Antiganglioside antibodies break this protective shield and allow complements to attach to neuronal cells easily and further cause massive cell injury. In addition, the deposition of antiganglioside antibodies forms the immune complexes, which cause inflammation and tissue damage, trigger leukocyte recruitment, augment antigen presentation, and activate the complement system. Furthermore, antiganglioside antibody–induced membrane structural changes alter the normal neuronal function that relies on the intact neuronal membrane (1, 60). Therefore, the B-cell immunity remains important in GBS regarding the pathogenic features of antiganglioside antibodies.

The Neurotrophic Factor BDNF in GBS

In our report, the BDNF level elevated consistently at the acute phase (before-treatment blood sampling) in all four cases. The member of the neurotrophin family, BDNF, involves in neuronal plasticity, survival, synaptogenesis, and neurotransmitters modulation (61). Even if BDNF is not a cytokine, increasing evidence has linked BDNF to neuroinflammation (22, 62). Although BDNF elevation signifies neuroinflammatory processes in brain disorders, its significance in peripheral nerve disorders is not fully understood.

During repairing peripheral nerve injury, the neurotrophins, particularly BDNF, serve for axon regeneration. *Via* signaling through cell surface tropomyosin receptor kinases (Trk) receptor and p75 neurotrophin receptor, two separate intracellular signaling pathways work for neuronal survival and neuronal plasticity (63). Increased expression of BDNF mRNA and TrkB mRNA in motor neurons suggests that BDNF responds to nerve injury (64). BDNF can be synthesized by dorsal root ganglion, as well in the circumstance of peripheral nerve inflammation (65). In lesioned peripheral nerves, Schwann cells dramatically increase the BDNF synthesis with a much higher amplitude than that of nerve growth factor (66).

Monoclonal antibodies are reliable in quantifying the blood concentration of BDNF (67). Many studies used BDNF level for clinical correlations or outcome predictions in various neurological and psychiatric diseases, such as Alzheimer disease (68, 69), Parkinson disease (70), Huntington disease (71), major depressive disorder (72), and multiple sclerosis (73, 74). BDNF augmentation was considered a potential disease-modifying strategy in neurodegenerative (75) and neuroinflammatory diseases (76). In inflammatory neuropathies, subcutaneous injection of BDNF had been tried on GBS patients to improve recovery (27); however, the results did not support its therapeutic use because of the small sample size, nonsignificant effects on improving disability after 4 weeks [mean difference, 0.75; 95% confidence interval, −1.14 to 2.64; very low certainty of the evidence (77)], and early termination of the trial. Although not being considered as a therapeutic agent, BDNF remains potential as a biomarker of the acute phase of GBS and warrants further studies.

Does Immunotherapy Affect Cytokine Levels?

Therapeutic apheresis, including plasma exchange and plasmapheresis, is an effective treatment of GBS (78). Plasma exchange is a centrifugation-based technique to separate patients'

blood components and replace them with fluid and plasma from healthy people. Plasmapheresis separates patients' plasma *via* a filtration-based device to remove large molecules such as antibodies and immune complexes and infuses the filtrated plasma back to the patients (79). Plasma exchange is theoretically able to remove more small molecules, such as cytokines, than plasmaspheres and results in a short-term benefit in improving the disability score of GBS; however, their long-term benefits to GBS patients do not differ (80). Currently, the argument of whether plasma exchange or plasmapheresis alters circulating cytokines remains inconclusive (81). Presumably, the intensity of plasma removal might matter, and only intensive plasma removal correlates with significant cytokine changes (82). The post–plasma exchange cytokine rebounding phenomenon might be another factor of the inconsistent results (83). Moreover, the mechanisms of action of plasma exchange and plasmapheresis are far more complex than merely removal of blood components. They may involve in the proliferation of normal B-cell population, correction of the imbalanced T_H1/T_H2 antagonism, and upregulation of suppressor T and Treg cells (79). Therefore, the cytokine changes we measured are the overall effects after therapeutic apheresis.

IVIG is another equally effective treatment of GBS (84). IVIG is suggested to achieve therapeutic effects in GBS *via* reduction of IL-1, intercellular adhesion molecule-1, and especially TNF-α, which is significantly higher in GBS than other neurological disease controls (85–87). The complex reaction after IVIG infusion also regards the increase of T-cell production of transforming growth factor β and upregulation of Fcγ receptor on B cells and monocytes (87).

Glucocorticoids strongly repress the immunomodulatory transcription factors, nuclear factor κB (NF-κB) and activator protein-1, to achieve therapeutic effects in autoimmune diseases (88, 89). The depression of NF-κB results in multiple immunosuppressing responses, including downregulation of proinflammatory cytokines, chemokine, and adhesion molecules and reduction of inflammatory T cells and macrophages (90). Circulating anti-inflammatory cytokines also increase in response to glucocorticoids (91).

To summarize, plasmapheresis, IVIG, and steroid treatment in our patients all may more or less affect their plasma cytokine levels *via* multiple immune mechanisms. The cytokine changes we measured after treatment are the net effects of disease recovery and treatment-related immune corrections. Therefore, overall considerations of GBS pathogenesis and immunotherapy effects and observations of multiple targets of immune systems are necessary for interpreting the before–after changes of cytokine levels.

Limitations of the Study

There were several limitations to this study. First, the small case number restricted its generalization to all GBS patients. The power of discussion on each single GBS variant or IgM/IG-related GBS cases was limited. Second, lacking a control group limited the statistical power of this study and restricted the generalizability of the results. Even if the before–after paired comparison is advantageous in highlighting the interindividual difference of immune responses, comparisons to a proper control group could objectively evaluate the value of these biomarkers.

Third, the interval between two blood sampling was not consistent among the patients. Although we arranged the second blood sample according to stabilization of individuals' conditions, the wide range of sampling intervals might raise considerations of multifactorial interferences to cytokine levels, such as environmental factors, underlying diseases, and acute stress responses. In contrast, the short between-test period might confound the results because patients might still be in the acute phase, and cytokine levels had not reached a plateau. A reasonable and fixed sampling time will help to overcome this limitation in future studies.

Finally, we limited the cytokine tests to the five representative cytokines because of the limited experimental resources, and it might not show the complete picture of disease mechanisms. Several commonly reported crucial cytokines, such as the IL-6, IL-10, interferon γ, and TNF-α, were not measured here. Choosing a group of cytokines per immune cell type will expand our knowledge of the immune mechanisms of GBS and have good use of the strength of the Luminex platform.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors under reasonable request, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of Chang Gung Medical Foundation, with approval number 201700701A3. Written informed consent to participate in this study was provided by the participants or the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

C-LW contributed to study conception, data analysis, and revision of the manuscript. C-HC drafted the manuscript. S-WL, Y-YC, W-YH, W-CW, and F-CS contributed to study design and data acquisition. Y-CW draft and revised the manuscript, collected data, and run laboratory analysis and interpretation. All authors have read and approved the manuscript.

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REFERENCES

1. Cutillo G, Saariaho AH, Meri S. Physiology of gangliosides and the role of antiganglioside antibodies in human diseases. *Cell Mol Immunol.* (2020) 17:313–22. doi: 10.1038/s41423-020-0388-9
2. Goodyear CS, O'Hanlon GM, Plomp JJ, Wagner ER, Morrison I, Veitch J, et al. Monoclonal antibodies raised against Guillain-Barré syndrome-associated Campylobacter jejuni lipopolysaccharides react with neuronal gangliosides and paralyze muscle-nerve preparations. *J Clin Invest.* (1999) 104:697–708. doi: 10.1172/JCI6837
3. McGonigal R, Rowan EG, Greenshields KN, Halstead SK, Humphreys PD, Rother RP, et al. Anti-GD1a antibodies activate complement and calpain to injure distal motor nodes of Ranvier in mice. *Brain.* (2010) 133:1944–60. doi: 10.1093/brain/awq119
4. Zhang G, Lehmann HC, Manoharan S, Hashmi M, Shim S, Ming GL, et al. Anti-ganglioside antibody-mediated activation of RhoA induces inhibition of neurite outgrowth. *J Neurosci.* (2011) 31:1664–75. doi: 10.1523/JNEUROSCI.3829-10.2011
5. Rupp A, Morrison I, Barrie JA, Halstead SK, Townson KH, Greenshields KN, et al. Motor nerve terminal destruction and regeneration following anti-ganglioside antibody and complement-mediated injury: an in and *ex vivo* imaging study in the mouse. *Exp Neurol.* (2012) 233:836–48. doi: 10.1016/j.expneurol.2011.12.010
6. Kaida K, Kanzaki M, Morita D, Kamakura K, Motoyoshi K, Hirakawa M, et al. Anti-ganglioside complex antibodies in Miller Fisher syndrome. *J Neurol Neurosurg Psychiatry.* (2006) 77:1043–6. doi: 10.1136/jnnp.2006.087940
7. Kaida K, Morita D, Kanzaki M, Kamakura K, Motoyoshi K, Hirakawa M, et al. Anti-ganglioside complex antibodies associated with severe disability in GBS. *J Neuroimmunol.* (2007) 182:212–8. doi: 10.1016/j.jneuroim.2006.09.013
8. Wang Y, Sun S, Zhu J, Cui L, Zhang HL. Biomarkers of Guillain-Barre syndrome: some recent progress, more still to be explored. *Mediators Inflamm.* (2015) 2015:564098. doi: 10.1155/2015/564098
9. Petzold A, Brettschneider J, Jin K, Keir G, Murray NM, Hirsch NP, et al. CSF protein biomarkers for proximal axonal damage improve prognostic accuracy in the acute phase of Guillain-Barre syndrome. *Muscle Nerve.* (2009) 40:42–9. doi: 10.1002/mus.21239
10. Fokkink WR, Walgaard C, Kuitwaard K, Tio-Gillen AP, Van Doorn PA, Jacobs BC. Association of albumin levels with outcome in intravenous immunoglobulin-treated guillain-barre syndrome. *JAMA Neurol.* (2017) 74:189–96. doi: 10.1001/jamaneurol.2016.4480
11. Altmann P, De Simoni D, Kaider A, Ludwig B, Rath J, Leutmezer F, et al. Increased serum neurofilament light chain concentration indicates poor outcome in Guillain-Barre syndrome. *J Neuroinflammation.* (2020) 17:86. doi: 10.1186/s12974-020-01737-0
12. Chun H-Y, Chung J-W, Kim H-A, Yun J-M, Jeon J-Y, Ye Y-M, et al. Cytokine IL-6 and IL-10 as biomarkers in systemic lupus erythematosus. *J Clin Immunol.* (2007) 27:461–6. doi: 10.1007/s10875-007-9104-0
13. Ragheb S, Lisak R, Lewis R, Van Stavern G, Gonzales F, Simon K. A potential role for B-cell activating factor in the pathogenesis of autoimmune myasthenia gravis. *Arch Neurol.* (2008) 65:1358–62. doi: 10.1001/archneur.65.10.1358
14. Kang SY, Kang CH, Lee KH. B-cell-activating factor is elevated in serum of patients with myasthenia gravis. *Muscle Nerve.* (2016) 54:1030–3. doi: 10.1002/mus.25162
15. Bick S, Tschernatsch M, Karg A, Fuehlhuber V, Trenczek TE, Faltermeier K, et al. Intravenous immunoglobulin inhibits BAFF production in chronic inflammatory demyelinating polyneuropathy - a new mechanism of action? *J Neuroimmunol.* (2013) 256:84–90. doi: 10.1016/j.jneuroim.2013.01.001
16. Ritter C, Förster D, Albrecht P, Hartung HP, Kieseier BC, Lehmann HC. IVIG regulates BAFF expression in patients with chronic inflammatory demyelinating polyneuropathy (CIDP). *J Neuroimmunol.* (2014) 274:225–9. doi: 10.1016/j.jneuroim.2014.06.007
17. Sun T, Chen X, Shi S, Liu Q, Cheng Y. Peripheral blood and cerebrospinal fluid cytokine levels in Guillain Barre syndrome: a systematic review and meta-analysis. *Front Neurosci.* (2019) 13:717. doi: 10.3389/fnins.2019.00717
18. Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. *Nat Rev Immunol.* (2002) 2:465–75. doi: 10.1038/nri844
19. Kataoka Y. Thymus and activation-regulated chemokine as a clinical biomarker in atopic dermatitis. *J Dermatol.* (2014) 41:221–9. doi: 10.1111/1346-8138.12440
20. Karnell JL, Rieder SA, Ettinger R, Kolbeck R. Targeting the CD40-CD40L pathway in autoimmune diseases: humoral immunity and beyond. *Adv Drug Deliv Rev.* (2019) 141:92–103. doi: 10.1016/j.addr.2018.12.005
21. Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: pathophysiological mechanisms. *J Immunol.* (2013) 190:3831–8. doi: 10.4049/jimmunol.1203487
22. Lima Giacobbo B, Doorduin J, Klein HC, Dierckx R, Bromberg E, De Vries EFJ. Brain-derived neurotrophic factor in brain disorders: focus on neuroinflammation. *Mol Neurobiol.* (2019) 56:3295–312. doi: 10.1007/s12035-018-1283-6
23. Khan SS, Smith MS, Reda D, Suffredini AF, Mccoy JP Jr. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufacturers. *Cytometry B Clin Cytom.* (2004) 61:35–9. doi: 10.1002/cyto.b.20021
24. Morgan E, Varro R, Sepulveda H, Ember JA, Apgar J, Wilson J, et al. Cytometric bead array: a multiplexed assay platform with applications in various areas of biology. *Clin Immunol.* (2004) 110:252–66. doi: 10.1016/j.clim.2003.11.017
25. Leonhard SE, Mandarakas MR, Gondim FA, Bateman K, Ferreira MLB, et al. Diagnosis and management of Guillain-Barre syndrome in ten steps. *Nat Rev Neurol.* (2019) 15:671–83. doi: 10.1038/s41582-019-0250-9
26. Chiba A, Kusunoki S, Obata H, Machinami R, Kanazawa I. Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: clinical and immunohistochemical studies. *Neurology.* (1993) 43:1911–7. doi: 10.1212/WNL.43.10.1911
27. Bensa S, Hadden RD, Hahn A, Hughes RA, Willison HJ. Randomized controlled trial of brain-derived neurotrophic factor in Guillain-Barre syndrome: a pilot study. *Eur J Neurol.* (2000) 7:423–6. doi: 10.1046/j.1468-1331.2000.00096.x
28. Wanschitz J, Maier H, Lassmann H, Budka H, Berger T. Distinct time pattern of complement activation and cytotoxic T cell response in Guillain-Barre syndrome. *Brain.* (2003) 126:2034–42. doi: 10.1093/brain/awg207
29. Misawa S, Kuwabara S, Sato Y, Yamaguchi N, Nagashima K, Katayama K, et al. Safety and efficacy of eculizumab in Guillain-Barré syndrome: a multicentre, double-blind, randomised phase 2 trial. *Lancet Neurol.* (2018) 17:519–29. doi: 10.1016/S1474-4422(18)30114-5
30. Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, Funakoshi K, et al. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. *J Neurosci.* (2007) 27:3956–67. doi: 10.1523/JNEUROSCI.4401-06.2007
31. Dalakas MC, Alexopoulos H, Spaeth PJ. Complement in neurological disorders and emerging complement-targeted therapeutics. *Nat Rev Neurol.* (2020) 16:601–17. doi: 10.1038/s41582-020-0400-0
32. Basta M, Illa I, Dalakas MC. Increased *in vitro* uptake of the complement C3b in the serum of patients with Guillain-Barré syndrome, myasthenia gravis and dermatomyositis. *J Neuroimmunol.* (1996) 71:227–9.
33. Hafer-Macko C, Sheikh K, Li C, Ho T, Cornblath D, Mckhann G, et al. Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Ann Neurol.* (1996) 39:625–35. doi: 10.1002/ana.410390512
34. He L, Zhang G, Liu W, Gao T, Sheikh KA. Anti-ganglioside antibodies induce nodal and axonal injury *via* fcgamma receptor-mediated inflammation. *J Neurosci.* (2015) 35:6770–85. doi: 10.1523/JNEUROSCI.4926-14.2015
35. Nyati KK, Prasad KN, Rizwan A, Verma A, Paliwal VK. TH1 and TH2 response to Campylobacter jejuni antigen in Guillain-Barre syndrome. *Arch Neurol.* (2011) 68:445–52. doi: 10.1001/archneurol.2011.51
36. Li S, Jin T, Zhang HL, Yu H, Meng F, Concha Quezada H, et al. Circulating Th17, Th22, and Th1 cells are elevated in the Guillain-Barre syndrome and downregulated by IVIg treatments. *Mediators Inflamm.* (2014) 2014:740947. doi: 10.1155/2014/740947
37. Chi LJ, Wang HB, Zhang Y, Wang WZ. Abnormality of circulating CD4(+)CD25(+) regulatory T cell in patients with Guillain-Barré syndrome. *J Neuroimmunol.* (2007) 192:206–14. doi: 10.1016/j.jneuroim.2007.09.034

38. Pritchard J, Makowska A, Gregson NA, Hayday AC, Hughes RA. Reduced circulating CD4+CD25+ cell populations in Guillain-Barré syndrome. *J Neuroimmunol.* (2007) 183:232–8. doi: 10.1016/j.jneuroim.2006.12.002
39. Wang FJ, Cui D, Qian WD. Therapeutic Effect of CD4+CD25+ regulatory T cells amplified *in vitro* on experimental autoimmune neuritis in rats. *Cell Physiol Biochem.* (2018) 47:390–402. doi: 10.1159/000489919
40. Dahle C, Vrethem M, Ernerudh J. T lymphocyte subset abnormalities in peripheral blood from patients with the Guillain-Barre syndrome. *J Neuroimmunol.* (1994) 53:219–25. doi: 10.1016/0165-5728(94)90032-9
41. Zhang HL, Zheng XY, Zhu J. Th1/Th2/Th17/Treg cytokines in Guillain-Barre syndrome and experimental autoimmune neuritis. *Cytokine Growth Factor Rev.* (2013) 24:443–53. doi: 10.1016/j.cytogfr.2013.05.005
42. Hou HQ, Miao J, Feng XD, Han M, Song XJ, Guo L. Changes in lymphocyte subsets in patients with Guillain-Barre syndrome treated with immunoglobulin. *BMC Neurol.* (2014) 14:202. doi: 10.1186/s12883-014-0202-3
43. Zhang Q, Vignali DA. Co-stimulatory and co-inhibitory pathways in autoimmunity. *Immunity.* (2016) 44:1034–51. doi: 10.1016/j.immuni.2016.04.017
44. Edner NM, Carlesso G, Rush JS, Walker LSK. Targeting co-stimulatory molecules in autoimmune disease. *Nat Rev Drug Discov.* (2020) 19:860–83. doi: 10.1038/s41573-020-0081-9
45. Wang YZ, Feng XG, Wang Q, Xing CY, Shi QG, Kong QX, et al. Increased plasmacytoid dendritic cells in Guillain-Barre syndrome. *J Neuroimmunol.* (2015) 283:1–6. doi: 10.1016/j.jneuroim.2015.03.019
46. Brunn A, Utermöhlen O, Mihelcic M, Saupe L, Fiocco Z, Schmidt A, et al. Costimulatory molecule CD40 is essential for myelin protein 0 peptide 106-125-induced experimental autoimmune neuritis in mice. *J Neuropathol Exp Neurol.* (2014) 73:454–66. doi: 10.1097/NEN.0000000000000069
47. Zhu W, Mix E, Jin T, Adem A, Zhu J. B cells play a cooperative role *via* CD40L-CD40 interaction in T cell-mediated experimental autoimmune neuritis in Lewis rats. *Neurobiol Dis.* (2007) 25:642–8. doi: 10.1016/j.nbd.2006.11.010
48. Kiefer R, Dangond F, Mueller M, Toyka KV, Hafler DA, Hartung HP. Enhanced B7 costimulatory molecule expression in inflammatory human sural nerve biopsies. *J Neurol Neurosurg Psychiatry.* (2000) 69:362–8. doi: 10.1136/jnnp.69.3.362
49. Hu W, Janke A, Ortler S, Hartung HP, Leder C, Kieseier BC, et al. Expression of CD28-related costimulatory molecule and its ligand in inflammatory neuropathies. *Neurology.* (2007) 68:277–82. doi: 10.1212/01.wnl.0000250240.99311.9d
50. Chiang S, Ubogu EE. The role of chemokines in Guillain-Barre syndrome. *Muscle Nerve.* (2013) 48:320–30. doi: 10.1002/mus.23829
51. Orlikowski D, Chazaud B, Plonquet A, Poron F, Sharshar T, Maison P, et al. Monocyte chemoattractant protein 1 and chemokine receptor CCR2 productions in Guillain-Barre syndrome and experimental autoimmune neuritis. *J Neuroimmunol.* (2003) 134:118–27. doi: 10.1016/S0165-5728(02)00393-4
52. Xia RH, Yosef N, Ubogu EE. Selective expression and cellular localization of pro-inflammatory chemokine ligand/receptor pairs in the sciatic nerves of a severe murine experimental autoimmune neuritis model of Guillain-Barre syndrome. *Neuropathol Appl Neurobiol.* (2010) 36:388–98. doi: 10.1111/j.1365-2990.2010.01092.x
53. Yuan F, Yosef N, Lakshmana Reddy C, Huang A, Chiang SC, Tithi HR, et al. CCR2 gene deletion and pharmacologic blockade ameliorate a severe murine experimental autoimmune neuritis model of Guillain-Barre syndrome. *PLoS One.* (2014) 9:e90463. doi: 10.1371/journal.pone.0090463
54. Duan RS, Chen Z, Bao L, Quezada HC, Nennesmo I, Winblad B, et al. CCR5 deficiency does not prevent P0 peptide 180-199 immunized mice from experimental autoimmune neuritis. *Neurobiol Dis.* (2004) 16:630–7. doi: 10.1016/j.nbd.2004.04.007
55. Kieseier BC, Tani M, Mahad D, Oka N, Ho T, Woodroofe N, et al. Chemokines and chemokine receptors in inflammatory demyelinating neuropathies: a central role for IP-10. *Brain.* (2002) 125:823–34. doi: 10.1093/brain/awf070
56. Scheu S, Ali S, Ruland C, Arolt V, Alferink J. The C-C chemokines CCL17 and CCL22 and their receptor CCR4 in CNS autoimmunity. *Int J Mol Sci.* (2017) 18:2306. doi: 10.3390/ijms18112306
57. Wang Q, Xing C, Hao Y, Shi Q, Qi Z, Lv Z, et al. Memory B cells in Guillain-Barre syndrome. *J Neuroimmunol.* (2017) 305:1–4. doi: 10.1016/j.jneuroim.2017.01.004
58. Willison HJ, O'Hanlon G, Paterson G, O'Leary CP, Veitch J, Wilson G, et al. Mechanisms of action of anti-GM1 and anti-GQ1b ganglioside antibodies in Guillain-Barre syndrome. *J Infect Dis.* (1997) 176(Suppl 2):S144–9. doi: 10.1086/513799
59. Yuki N. Guillain-Barre syndrome and anti-ganglioside antibodies: a clinician-scientist's journey. *Proc Jpn Acad Ser B Phys Biol Sci.* (2012) 88:299–326. doi: 10.2183/pjab.88.299
60. Kaida K, Ariga T, Yu RK. Antiganglioside antibodies and their pathophysiological effects on Guillain-Barre syndrome and related disorders–a review. *Glycobiology.* (2009) 19:676–92. doi: 10.1093/glycob/cwp027
61. Edelmann E, Lessmann V, Brigadski TJ. Pre-and postsynaptic twists in BDNF secretion and action in synaptic plasticity. *Neuropharmacology.* (2014) 76:610–27. doi: 10.1016/j.neuropharm.2013.05.043
62. Calabrese F, Rossetti AC, Racagni G, Gass P, Riva MA, Molteni R. Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. *Front Cell Neurosci.* (2014) 8:430. doi: 10.3389/fncel.2014.00430
63. Fayard B, Loeffler S, Weis J, Vögelin E, Krüttgen AJJONR. The secreted brain-derived neurotrophic factor precursor pro-BDNF binds to TrkB and p75NTR but not to TrkA or TrkC. *J Neurosci Res.* (2005) 80:18–28. doi: 10.1002/jnr.20432
64. Gordon T. The role of neurotrophic factors in nerve regeneration. *Neurosurg Focus.* (2009) 26:E3. doi: 10.3171/FOC.2009.26.2.E3
65. Cho H-J, Kim S-Y, Park M-J, Kim D-S, Kim J-K, Chu M-Y. Expression of mRNA for brain-derived neurotrophic factor in the dorsal root ganglion following peripheral inflammation. *Brain Res.* (1997) 749:358–62. doi: 10.1016/S0006-8993(97)00048-6
66. Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J Cell Biol.* (1992) 119:45–54. doi: 10.1083/jcb.119.1.45
67. Naegelin Y, Dingsdale H, Sauberli K, Schadelin S, Kappos L, Barde YA. Measuring and validating the levels of brain-derived neurotrophic factor in human serum. *eNeuro.* (2018) 5(2):ENEURO.0419-17.2018. doi: 10.1523/ENEURO.0419-17.2018
68. Laske C, Stransky E, Leyhe T, Eschweiler GW, Schott K, Langer H, et al. Decreased brain-derived neurotrophic factor (BDNF)- and beta-thromboglobulin (beta-TG)- blood levels in Alzheimer's disease. *Thromb Haemost.* (2006) 96:102–3. doi: 10.1160/TH06-03-0173
69. Laske C, Stellos K, Hoffmann N, Stransky E, Straten G, Eschweiler GW, et al. Higher BDNF serum levels predict slower cognitive decline in Alzheimer's disease patients. *Int J Neuropsychopharmacol.* (2011) 14:399–404. doi: 10.1017/S1461145710001008
70. Jiang L, Zhang H, Wang C, Ming F, Shi X, Yang M. Serum level of brain-derived neurotrophic factor in Parkinson's disease: a meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry.* (2019) 88:168–74. doi: 10.1016/j.pnpbp.2018.07.010
71. Ciammola A, Sassone J, Cannella M, Calza S, Poletti B, Frati L, et al. Low brain-derived neurotrophic factor (BDNF) levels in serum of Huntington's disease patients. *Am J Med Genet B Neuropsychiatr Genet.* (2007) 144B:574–7. doi: 10.1002/ajmg.b.30501
72. Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol.* (2008) 11:1169–80. doi: 10.1017/S1461145708009309
73. Patanella AK, Zinno M, Quaranta D, Nociti V, Frisullo G, Gainotti G, et al. Correlations between peripheral blood mononuclear cell production of BDNF, TNF-alpha, IL-6, IL-10 and cognitive performances in multiple sclerosis patients. *J Neurosci Res.* (2010) 88:1106–12. doi: 10.1002/jnr.22276
74. Yoshimura S, Ochi H, Isobe N, Matsushita T, Motomura K, Matsuoka T, et al. Altered production of brain-derived neurotrophic factor by peripheral blood immune cells in multiple sclerosis. *Mult Scler.* (2010) 16:1178–88. doi: 10.1177/1352458510375706

75. Lu B, Nagappan G, Guan X, Nathan PJ, Wren P. BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat Rev Neurosci.* (2013) 14:401–16. doi: 10.1038/nrn3505
76. Linker RA, Lee DH, Demir S, Wiese S, Kruse N, Siglienti I, et al. Functional role of brain-derived neurotrophic factor in neuroprotective autoimmunity: therapeutic implications in a model of multiple sclerosis. *Brain.* (2010) 133:2248–63. doi: 10.1093/brain/awq179
77. Doets AY, Hughes RA, Brassington R, Hadden RD, Pritchard J. Pharmacological treatment other than corticosteroids, intravenous immunoglobulin and plasma exchange for Guillain-Barre syndrome. *Cochrane Database Syst Rev.* (2020) 1:CD008630. doi: 10.1002/14651858.CD008630.pub5
78. Chevret S, Hughes RA, Annane D. Plasma exchange for Guillain-Barre syndrome. *Cochrane Database Syst Rev.* (2017) 2:CD001798. doi: 10.1002/14651858.CD001798.pub3
79. Reeves HM, Winters JL. The mechanisms of action of plasma exchange. *Br J Haematol.* (2014) 164:342–51. doi: 10.1111/bjh.12629
80. Lyu RK, Chen WH, Hsieh ST. Plasma exchange versus double filtration plasmapheresis in the treatment of Guillain-Barre syndrome. *Ther Apher.* (2002) 6:163–6. doi: 10.1046/j.1526-0968.2002.00382.x
81. Yeh JH, Wang SH, Chien PJ, Shih CM, Chiu HC. Changes in serum cytokine levels during plasmapheresis in patients with myasthenia gravis. *Eur J Neurol.* (2009) 16:1318–22. doi: 10.1111/j.1468-1331.2009.02729.x
82. Nakae H, Asanuma Y, Tajimi K. Cytokine removal by plasma exchange with continuous hemodiafiltration in critically ill patients. *Ther Apher.* (2002) 6:419–24. doi: 10.1046/j.1526-0968.2002.00464.x
83. Shariatmadar S, Nassiri M, Vincek V. Effect of plasma exchange on cytokines measured by multianalyte bead array in thrombotic thrombocytopenic purpura. *Am J Hematol.* (2005) 79:83–8. doi: 10.1002/ajh.20342
84. Hughes RA, Swan AV, Van Doorn PA. Intravenous immunoglobulin for Guillain-Barre syndrome. *Cochrane Database Syst Rev.* (2014) 2014:CD002063. doi: 10.1002/14651858.CD002063.pub6
85. Exley AR, Smith N, Winer JB. Tumour necrosis factor-alpha and other cytokines in Guillain-Barré syndrome. *J Neurol Neurosurg Psychiatry.* (1994) 57:1118–20. doi: 10.1136/jnnp.57.9.1118
86. Sharief MK, Ingram DA, Swash M, Thompson EJ. I.v. immunoglobulin reduces circulating proinflammatory cytokines in Guillain-Barre syndrome. *Neurology.* (1999) 52:1833–8. doi: 10.1212/WNL.52.9.1833
87. Lehmann HC, Hartung HP. Plasma exchange and intravenous immunoglobulins: mechanism of action in immune-mediated neuropathies. *J Neuroimmunol.* (2011) 231:61–9. doi: 10.1016/j.jneuroim.2010.09.015
88. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol.* (2011) 335:2–13. doi: 10.1016/j.mce.2010.04.005
89. Strehl C, Ehlers L, Gaber T, Buttgereit F. Glucocorticoids-all-rounders tackling the versatile players of the immune system. *Front Immunol.* (2019) 10:1744. doi: 10.3389/fimmu.2019.01744
90. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther.* (2017) 2:17023. doi: 10.1038/sigtrans.2017.23
91. Richards DF, Fernandez M, Caulfield J, Hawrylowicz CM. Glucocorticoids drive human CD8(+) T cell differentiation towards a phenotype with high IL-10 and reduced IL-4, IL-5 and IL-13 production. *Eur J Immunol.* (2000) 30:2344–54. doi: 10.1002/1521-4141(2000)30:8<2344::AID-IMMU2344>3.0.CO;2-7

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CASE REPORT
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Posterior Interosseous Fascicular Constriction Within the Radial Nerve in a Diabetic Patient With Bilateral Neuralgic Amyotrophy: A Case Report

Woojun Kim[1], *Soo Hwan Kang*[2] *and Jae Young An*[3]*

[1] Department of Neurology, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, South Korea, [2] Department of Orthopedic Surgery, College of Medicine, St. Vincent's Hospital, The Catholic University of Korea, Seoul, South Korea, [3] Department of Neurology, College of Medicine, St. Vincent's Hospital, The Catholic University of Korea, Seoul, South Korea

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Jens Schmidt,
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Shunsuke Kobayashi,
Teikyo University, Japan

***Correspondence:**
Jae Young An
nrjyan@gmail.com

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Background: Neuralgic amyotrophy (NA) is an acute, monophasic, painful inflammatory dysimmune focal, or multifocal mononeuropathy. The lesion in NA is not always restricted to the brachial plexus but also involves individual nerves or branches. The prognosis of NA is less favorable than previously assumed, but the reasons for poor recovery remain unknown. Nerve constriction may be one of the causes of poor prognosis in NA.

Case Presentation: Herein, we described a 54-year-old male with a history of type 2 diabetes in whom bilateral neuralgic amyotrophy developed with constriction of the posterior interosseous fascicle within the radial nerve. The patient experienced sudden-onset severe pain in both shoulders followed, 2 days later, by weakness in bilateral shoulders and the left forearm extensors over the subsequent month. The left forearm extensors were more severely affected than both shoulder girdle muscles. He noted a 7-kg weight loss for 1 month before pain onset. After diagnosing diabetic NA based on the clinical symptoms, imaging, and electrophysiological studies, treatment with systemic steroids improved pain and weakness in both shoulder muscles. Weakness in the left forearm extensors persisted after 1 month of steroid treatment. Follow-up ultrasound revealed constriction of the posterior interosseous fascicle within the main trunk of the left radial nerve at the elbow. Surgical exploration at 6 months after onset identified fascicle constriction, for which neurolysis was performed. Weakness in the extensors of the wrist and fingers did not improve during the 16-month follow-up.

Conclusion: A single constriction of the fascicle within a peripheral nerve may often be under-recognized if NA presents with variable degrees of weakness in bilateral upper limbs. Furthermore, fascicular constriction without edema of the parent nerve may be easily missed on the initial ultrasound. A lack of early recognition of nerve constriction and delay in surgical intervention can result in unfavorable outcomes. The physician should

consider the possibility of the fascicular constriction when evaluating patients suspected of brachial NA with significant weakness in the distal upper limb compared to the proximal weakness or weakness of the distal upper limb that does not improve over time.

Keywords: ultrasound, fascicular constriction, posterior interosseous nerve, radial nerve, neuralgic amyotrophy

INTRODUCTION

Idiopathic neuralgic amyotrophy (NA), also known as Parsonage–Turner syndrome or brachial plexus neuritis, is characterized by extreme pain at symptom onset, rapid multifocal paresis, atrophy of the upper extremity muscles, and a slow recovery requiring months to years (1). Although the classical presentation is found in about two-thirds of patients, NA can also manifest with the involvement of a single peripheral nerve or various combinations of peripheral nerves of the brachial plexus (2). This clinical variation that also involves the nerve of non-brachial plexus origin, such as accessory nerve, has led to the concept of NA that encompasses all these acute-onset, painful focal, or multifocal neuropathies with a monophasic course (1–3). The prognosis of NA was traditionally thought to be favorable, with pain subsided within a few weeks and weakness improved over months to years. However, based on a large series of patients with NA, overall recovery was less favorable than previously assumed (4). The exact cause of the unfavorable outcome is unknown. However, previous studies reported surgical findings of nerve constriction in patients with typical symptoms of NA and no spontaneous recovery (5, 6).

Here, we report a case of a diabetic male with bilateral brachial NA showing posterior interosseous fascicular constriction within the radial nerve on ultrasound, and which was confirmed by surgical exploration.

CASE PRESENTATION

A 54-year-old man was admitted with a 2-month history of pain and weakness in both shoulders. The patient was an office worker with no past medical history except for well-controlled hypertension and diabetes for 10 years. Two months prior, he complained of sudden onset of pain in both shoulders and developed the weakness of both shoulder girdles 2 days later. The weakness in the left shoulder girdle progressed, and weakness in the left wrist and finger extensors newly developed over the next 4 weeks. There were no preceding trauma, immunization, or fever, but he reported weight loss (7 kg) over the 1 month before pain onset. The patient had never regularly exercised other than walking for glycemic control. The symptoms did not change for the next 1 month before admitting to our hospital. A neurological examination showed paresis of shoulder abduction [right: medical research council grade (MRC) 4/5, left: MRC 3/5], elbow flexion (right: MRC 4/5, left: MRC 3/5), elbow extension (right: MRC 4/5, left: MRC 3/5), wrist flexion (right: MRC 4/5, left: MRC 4/5), wrist extension (right: MRC 4/5, left: MRC 2/5), and extension of fingers (right: MRC 4/5, left: MRC 2/5). The muscle power of bilateral finger flexion and lower limbs was normal. A hypesthesia area was identified in the lateral sides of bilateral shoulders and arms, and deep tendon reflexes were reduced in the upper limbs. Atrophy of both shoulder girdles was observed (**Figure 1**). A complete blood cell count and routine biochemical analysis were normal except for fasting glucose (151 mg/dl) and HbA1c (7.3%). Other serological tests for human immunodeficiency virus, hepatitis B, C, and E, syphilis, and autoimmune diseases including angiotensin-converting enzyme, anti-nuclear antibody, double-stranded DNA, anti-Ro, anti-La, and anti-neutrophil cytoplasmic antibody were negative. An initial nerve conduction study (NCS), performed 2 months after symptom onset, showed a decrease of compound muscle action potential amplitude in the left musculocutaneous nerve (**Figure 2A**, recording of the biceps brachii muscle) and axillary nerve (**Figure 2B**, recording of the deltoid muscle) and a significant asymmetry of sensory nerve action potentials of the left lateral antebrachial cutaneous nerve (2.0 μV) compared to the right side (5.0 μV). NCS findings of the bilateral median, ulnar, peroneal, tibial, and sural nerves were normal. Needle electromyography (EMG) showed various degrees of denervation potentials in bilateral infraspinatus, supraspinatus, left deltoid, left triceps, left extensor digitorum, and left flexor carpi radialis muscles. There was reduced recruitment of motor unit in bilateral serratus anterior and right deltoid muscles. The results of initial electrophysiological studies suggested the involvement of bilateral upper and middle trunks (C5–C7) of the brachial plexus, particularly a more severe involvement of the left brachial plexus. Ultrasound with a 18–6-MHz linear transducer was performed on the same day as NCS/EMG and showed no swelling in the brachial plexus, median, radial, ulnar nerves. Magnetic resonance imaging of the brachial plexus was carried out 3 days after NCS/EMG, showing mild swelling without enhancement at the cord level of the left brachial plexus. Based on the clinical history of the acute intense pain of shoulders and weakness of upper limbs with preceding weight loss, considering the involvement of bilateral upper and middle branches of brachial plexus in the electrophysiological studies and having excluded other causes by imaging and serological studies, a diagnosis of diabetic NA was made. Oral steroid (1 mg/kg) was administered 9 weeks after symptom onset and tapered over 4 weeks. Bilateral shoulder pain and weakness, except for weakness of the left wrist and finger extensors, improved. In the follow-up study of the left axillary (**Figure 2C**) and musculocutaneous (**Figure 2D**) nerves 4 weeks after the initial study, the amplitude of compound muscle action potential was improved. However, on the conduction study of the left radial nerve that was not included in the initial study, we detected a conduction block between the spiral groove and the elbow (**Figure 2E**, recording of the extensor indicis muscle).

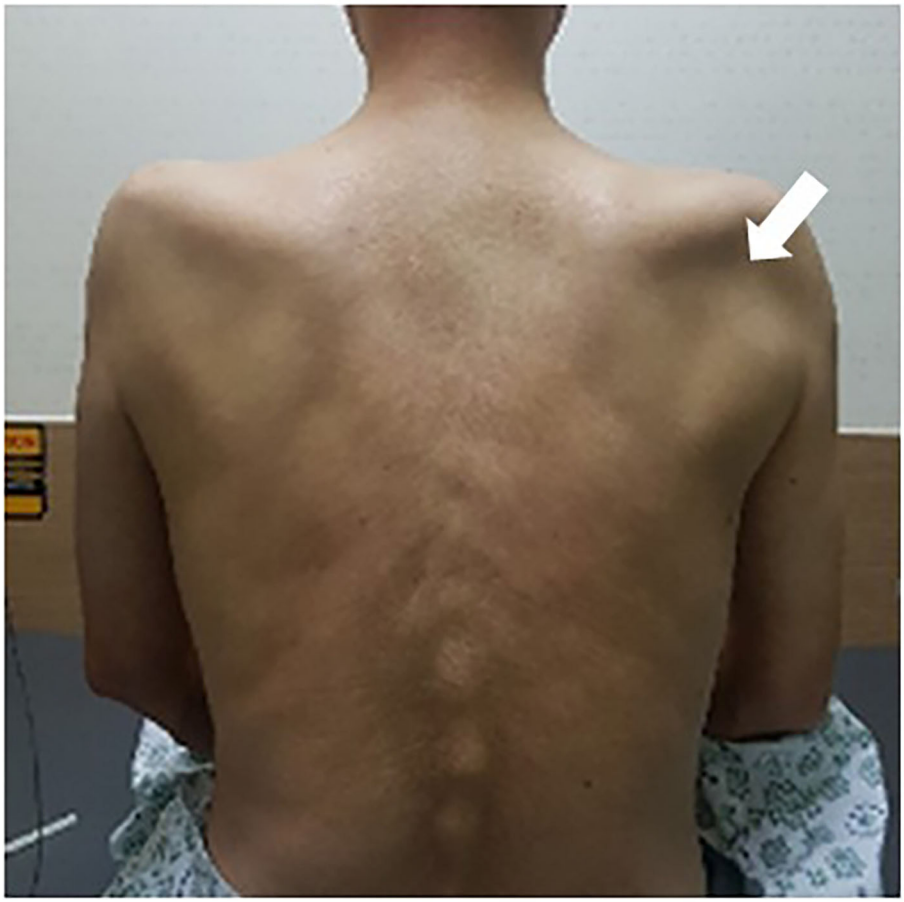

FIGURE 1 | Muscle atrophy of bilateral supraspinatus and infraspinatus. The bilateral infraspinatus fossae are dented, with greater severity on the right side (arrow).

The nerve action potentials of bilateral superficial radial sensory nerves were symmetric. A 4-week follow-up ultrasound revealed constriction of the left posterior interosseous fascicle within the left radial nerve at 1.5 cm proximal to the lateral epicondyle (**Figures 3A–D**). Surgical exploration for nerve constriction was considered but refused by the patient. Therefore, low-dose oral steroids and a rehabilitation program were continued for another 2 months. A 5-month follow-up NCS revealed a persistent conduction block of the left radial nerve (**Figure 2F**). The weakness of the left wrist and finger extensors was unchanged (MRC 2/5), but other remaining upper limb muscles were normal, except for the left shoulder abduction (MRC 4/5), left elbow flexion (MRC 4/5), and extension (MRC 4/5). Surgical exploration was performed 6 months after symptom onset and confirmed fascicular constriction at the area identified by ultrasound, for which interfascicular neurolysis was performed (**Figures 3E,F**). During the 16-month follow-up, the weakness in the wrist and finger extensors did not improve (MRC 2/5). We considered a tendon transfer to improve hand function a year after neurolysis, but the patient did not want secondary surgery.

DISCUSSION

Brachial NA remains unfamiliar to many physicians who often diagnose such patients with glenohumeral bursitis or cervical radiculopathy. Such a delay in diagnosis of NA can lead to suboptimal treatment in the acute stage (2, 4). The minimum incidence of NA is one to three cases per 100,000 individuals in the general population (7, 8); however, under-recognition and misdiagnosis are frequent, and the incidence recently has been estimated to be 1 per 1,000 per year (9). The precise pathophysiological mechanism remains unclear but is thought to result from an underlying genetic predisposition, a susceptibility to mechanical injury, and immune or autoimmune triggers for the attacks (2). Patients with diabetes mellitus sometimes present with acute or subacute, progressive, asymmetrical pain, and weakness of the proximal lower limb muscles, known as diabetic amyotrophy, Bruns-Garland syndrome, or diabetic lumbosacral radiculoplexus neuropathy (RPN) (10). Diabetic RPN can affect the upper limb, which shares many clinical features with NA (11). Massie et al. reported that diabetic cervical RPN had autonomic features, more frequent weight loss, co-occurring thoracic and

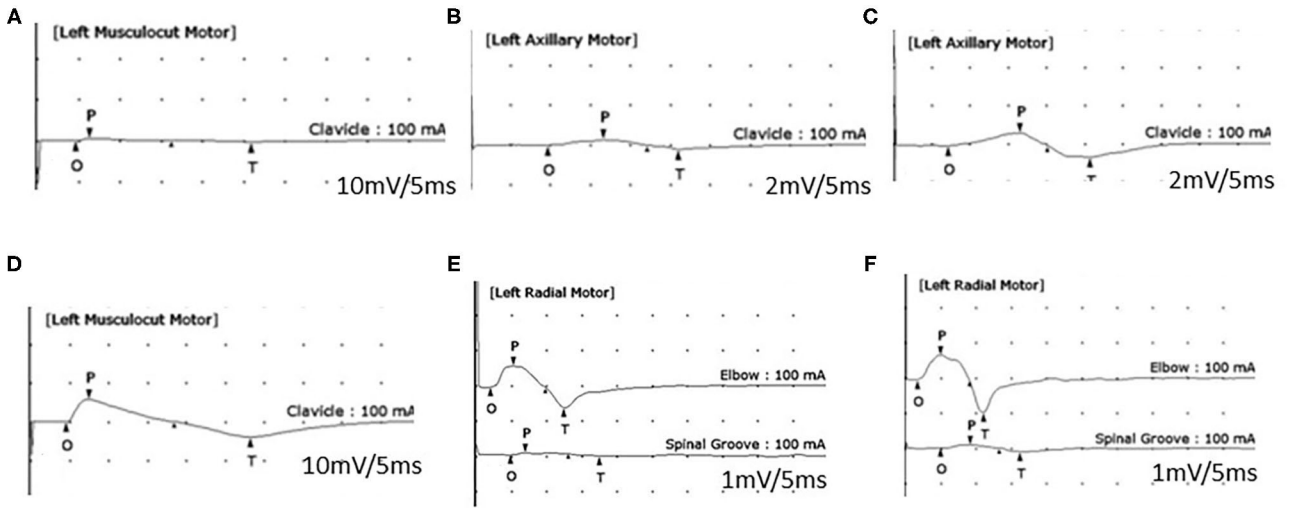

FIGURE 2 | Motor nerve conduction study (NCS). An initial NCS **(A,B)** shows a reduced amplitude of compound muscle action potentials (CMAPs) of the left musculocutaneous nerve (**A**: 1.1 mV, recording of the biceps brachii muscle) and left axillary nerve (**B**: 0.5 mV, recording of the deltoid muscle). A 1-month follow-up NCS **(C–E)** shows the improved amplitude of CMAPs of the left axillary nerve (**C**: 10.2 mV), the left musculocutaneous nerve (**D**: 13 mV), and a conduction block of the left radial nerve (recording of the extensor indicis muscle) between the elbow and spiral groove **(E)**. A 5-month follow-up NCS **(F)** reveals a persistent conduction block of the left radial nerve.

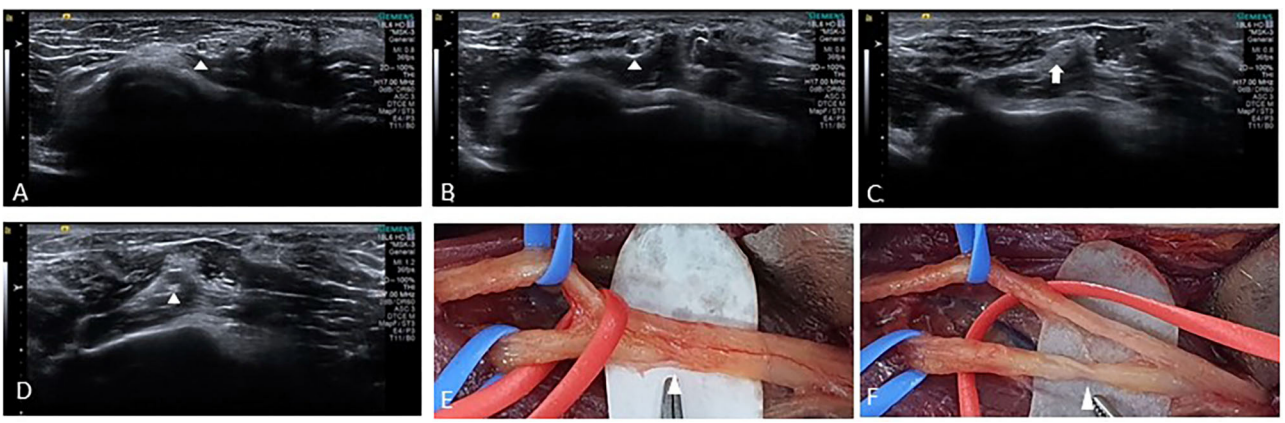

FIGURE 3 | Ultrasound images from cine clips of the left radial nerve in the short axis show a normal posterior interosseous fascicle (arrowhead) within the radial nerve **(A)**, swelling (arrowhead) is noted as the nerve is traced proximally **(B)**, followed by fascicular constriction (arrow) **(C)**, and a swollen fascicle (arrowhead) proximal to the constriction **(D)**. Intraoperative photograph shows partial constriction **(E)** of radial nerve before branching posterior interosseous and superficial radial sensory nerves proximal to the lateral epicondyle. After neurolysis, constriction **(F)** of the posterior interosseous fascicle was discovered.

lumbosacral RPN, more involvement outside of the brachial plexus, and more involvement of the lower trunk of the brachial plexus (11). However, other researchers suggested that NA in diabetic patients is merely a chance occurrence (2, 4). Recent studies suggested that NA and diabetic RPN are variants of non-systemic vasculitic neuropathies, but whether diabetic NA should be separated from idiopathic NA is a matter of ongoing debate (12, 13).

In several recent studies, persistent pain and paresis were experienced by up to two-thirds of patients with idiopathic NA (1, 2). Since first reported by Abe et al. (14) nerve constriction has been established as an unexplained surgical finding in various nerves of the upper extremity (15). Pan et al. first reported a surgical finding of hourglass-like constriction of the upper-extremity nerves in patients with typical symptoms of NA and no spontaneous recovery (6). Surgical treatment resulted in generally good recovery, and this nerve constriction or nerve torsion may be a clue for poor prognosis in some patients with NA (3, 15). The pathogenesis of nerve constriction and torsion is unclear but is considered due to local inflammation, which can cause intrafascicular edema, adhesion, and local fixation of fascicles, resulting in the thinning and constriction of nerve and making the nerve susceptible to torsion (5, 16, 17).

NA has been considered a predominantly clinical diagnosis, based on the characteristic history and physical findings, (1, 4) and laboratory tests are of little diagnostic value (2). Many clinicians are generally considering electrophysiological studies as the first test to diagnose brachial NA. However, the sensitivity of NCS for this disorder is very low, and EMG commonly produces negative results due to sampling error (18). With the introduction of improved imaging methods such as magnetic resonance imaging and high-resolution ultrasound in the diagnostic workup of NA, distinct structural nerve pathologies have been identified as pathognomonic in NA patients (5, 6, 15, 19, 20). Arányi et al. identified abnormal ultrasound findings in 74% of patients with NA (3). In their report, four types of abnormalities were classified as swelling without constriction, swelling with incomplete constriction, swelling with complete constriction, and fascicular entwinement. Nerve swelling is recognized easily on ultrasound, irrespective of constriction, and so is rotational nerve torsion. However, fascicular constriction within the parent nerve can be missed in the ultrasound without high-index suspicion.

Although it is still controversial when surgical treatment should be performed, surgical treatment should be considered for patients who did not show spontaneous recovery by 3 months after onset (5, 12, 21–23). Furthermore, if severe constrictions with nerve torsion and fascicular entwinement are identified through ultrasound before the 3-month interval, early intervention may be justified as spontaneous recovery is not to be expected in these cases (3). The choice of optimal surgical treatment for the patient with nerve constriction depends on the age, delay repair interval, and severity of constriction (23, 24). Surgical treatments include interfascicular neurolysis, tendon transfer, and neurorrhaphy or autografting. Intrafascicular neurolysis is suggested for mild to moderate constriction and nerve reconstruction for severe constriction (12, 23). Previous studies have noted that younger patients had a higher chance for good recovery, while patients aged 50 or older more frequently showed unfavorable results (12, 23, 24). Moreover, Ochi et al. suggested interfascicular neurolysis together with tendon transfer for patients over 50 years old with no sign of recovery or the younger group with a delay repair interval of more than a year (24).

The development of nerve constriction irrespective of torsion in brachial NA might be a sign of poor prognosis (15). We did not include radial nerve in the initial NCS, as we thought that asymmetric brachial plexus lesions caused prominent weakness of the left forearm extensors at the initial examination, and we were belatedly aware of the left radial nerve lesion due to persistent weakness of the left forearm extensors. Furthermore, the initial ultrasound was unable to identify fascicular constriction because fascicular constriction occurred in short segments without edema of the main trunk of the radial nerve. Even if we cannot determine which of the delay of the surgery, old age of the patient, or method of surgical treatment affected the poor prognosis through our single case, early detection of fascicular constriction is the essential step in optimizing the management of NA patients with nerve constriction. Therefore, a careful ultrasound examination needs to be performed in the initial evaluation for NA, and follow-up ultrasound for fascicular constriction should be considered if prominent distal weakness is present compared with proximal weakness or if there is the distal weakness that does not improve after treatment.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The catholic university of Korea, St. Vincent's Hospital institutional Review Board. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

WK, SK, and JA take responsibility of the study concept, and design, integrity of the data, drafting of the manuscript, and study supervision. SK and JA collected and analyzed patient information. All the authors read and approved the manuscript.

REFERENCES

1. van Alfen N. Clinical and pathophysiological concepts of neuralgic amyotrophy. *Nat Rev Neurol.* (2011) 7:315–22. doi: 10.1038/nrneurol.2011.62
2. Van Eijk JJ, Groothuis JT, Van Alfen N. Neuralgic amyotrophy: an update on diagnosis, pathophysiology, and treatment. *Muscle Nerve.* (2016) 53:337–50. doi: 10.1002/mus.25008
3. ArÁnyi Z, Csillik A, DéVay K, Rosero M, Barsi P, BÖhm J, et al. Ultrasonography in neuralgic amyotrophy: sensitivity, spectrum of findings, and clinical correlations. *Muscle Nerve.* (2017) 56:1054–62. doi: 10.1002/mus.25708
4. van Alfen N, van Engelen BG. The clinical spectrum of neuralgic amyotrophy in 246 cases. *Brain.* (2006) 129:438–50. doi: 10.1093/brain/awh722
5. Pan Y, Wang S, Zheng D, Tian W, Tian G, Ho PC, et al. Hourglass-like constrictions of peripheral nerve in the upper extremity: a clinical review and pathological study. *Neurosurgery.* (2014) 75:10–22. doi: 10.1227/NEU.0000000000000350
6. Pan YW, Wang S, Tian G, Li C, Tian W, Tian M. Typical brachial neuritis (Parsonage-Turner syndrome) with hourglass-like constrictions in the affected nerves. *J Hand Surg Am.* (2011) 36:1197–203. doi: 10.1016/j.jhsa.2011.03.041
7. Beghi E, Kurland LT, Mulder DW, Nicolosi A. Brachial plexus neuropathy in the population of Rochester, Minnesota, 1970-1981. *Ann Neurol.* (1985) 18:320–3. doi: 10.1002/ana.410180308
8. MacDonald BK, Cockerell OC, Sander JW, Shorvon SD. The incidence and lifetime prevalence of neurological disorders in

a prospective community-based study in the UK. *Brain.* (2000) 123:665–76. doi: 10.1093/brain/123.4.665
9. van Alfen N, van Eijk JJ, Ennik T, Flynn SO, Nobacht IE, Groothuis JT, et al. Incidence of neuralgic amyotrophy (Parsonage Turner syndrome) in a primary care setting–a prospective cohort study. *PLoS ONE.* (2015) 10:e0128361. doi: 10.1371/journal.pone.0128361
10. Chan YC, Lo YL, Chan ES. Immunotherapy for diabetic amyotrophy. *Cochrane Database Syst Rev.* (2017) 7:Cd006521. doi: 10.1002/14651858.CD006521.pub4
11. Massie R, Mauermann ML, Staff NP, Amrami KK, Mandrekar JN, Dyck PJ, et al. Diabetic cervical radiculoplexus neuropathy: a distinct syndrome expanding the spectrum of diabetic radiculoplexus neuropathies. *Brain.* (2012) 135:3074–88. doi: 10.1093/brain/aws244
12. Gstoettner C, Mayer JA, Rassam S, Hruby LA, Salminger S, Sturma A, et al. Neuralgic amyotrophy: a paradigm shift in diagnosis and treatment. *J Neurol Neurosurg Psychiatry.* (2020) 91:879–88. doi: 10.1136/jnnp-2020-323164
13. Collins MP, Dyck PJB, Hadden RDM. Update on classification, epidemiology, clinical phenotype and imaging of the nonsystemic vasculitic neuropathies. *Curr Opin Neurol.* (2019) 32:684–95. doi: 10.1097/WCO.0000000000000727
14. T Abe MH, N Shinohara, T Takamatsu. Isolated paralysis of the deep branch of the radial nerve thought to be the entrapment neuropathy (in Japanese). *Rinsho Seikei Geka.* (1966) 1:617–21.
15. Arányi Z, Csillik A, Dévay K, Rosero M, Barsi P, Böhm J, et al. Ultrasonographic identification of nerve pathology in neuralgic amyotrophy: enlargement, constriction, fascicular entwinement, and torsion. *Muscle Nerve.* (2015) 52:503–11. doi: 10.1002/mus.24615
16. Kotani H, Miki T, Senzoku F, Nakagawa Y, Ueo T. Posterior interosseous nerve paralysis with multiple constrictions. *J Hand Surg Am.* (1995) 20:15–17. doi: 10.1016/S0363-5023(05)80049-8
17. Endo Y, Miller TT, Carlson E, Wolfe SW. Spontaneous nerve torsion: unusual cause of radial nerve palsy. *Skeletal Radiol.* (2015) 44:457–61. doi: 10.1007/s00256-014-2006-3
18. van Alfen N, Huisman WJ, Overeem S, van Engelen BG, Zwarts MJ. Sensory nerve conduction studies in neuralgic amyotrophy. *Am J Phys Med Rehabil.* (2009) 88:941–6. doi: 10.1097/PHM.0b013e3181a5b980
19. Sneag DB, Rancy SK, Wolfe SW, Lee SC, Kalia V, Lee SK, et al. Brachial plexitis or neuritis? MRI features of lesion distribution in Parsonage-Turner syndrome. *Muscle Nerve.* (2018) 58:359–66. doi: 10.1002/mus.26108
20. Sneag DB, Saltzman EB, Meister DW, Feinberg JH, Lee SK, Wolfe SW. MRI bullseye sign: an indicator of peripheral nerve constriction in parsonage-turner syndrome. *Muscle Nerve.* (2017) 56:99–106. doi: 10.1002/mus.25480
21. Guerra WK, Schroeder HW. Peripheral nerve palsy by torsional nerve injury. *Neurosurgery.* (2011) 68:1018–24. discussion 1024. doi: 10.1227/NEU.0b013e31820a548c
22. Nagano A, Shibata K, Tokimura H, Yamamoto S, Tajiri Y. Spontaneous anterior interosseous nerve palsy with hourglass-like fascicular constriction within the main trunk of the median nerve. *J Hand Surg Am.* (1996) 21:266–70. doi: 10.1016/S0363-5023(96)80114-6
23. Wu P, Yang JY, Chen L, Yu C. Surgical and conservative treatments of complete spontaneous posterior interosseous nerve palsy with hourglass-like fascicular constrictions: a retrospective study of 41 cases. *Neurosurgery.* (2014) 75:250–7; discussion 257. doi: 10.1227/NEU.0000000000000424
24. Ochi K, Horiuchi Y, Tazaki K, Takayama S, Nakamura T, Ikegami H, et al. Surgical treatment of spontaneous posterior interosseous nerve palsy: a retrospective study of 50 cases. *J Bone Joint Surg Br.* (2011) 93:217–22. doi: 10.1302/0301-620X.93B2.24748

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CASE REPORT
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Case Report: Laryngospasm as Initial Manifestation of Amyotrophic Lateral Sclerosis in a Long-Survival Patient With Heterozygous p.D90A – *SOD1* Mutation

Giuliana Capece[1], *Mauro Ceroni*[1,2], *Enrico Alfonsi*[3], *Ilaria Palmieri*[4,5], *Cristina Cereda*[4]* *and Luca Diamanti*[6]

[1] Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy, [2] General Neurology Unit, IRCCS Mondino Foundation, Pavia, Italy, [3] Clinical Neurophysiology Unit, IRCCS Mondino Foundation, Pavia, Italy, [4] Genomic and Post-genomic Centre, IRCCS Mondino Foundation, Pavia, Italy, [5] Department of Molecular Medicine, University of Pavia, Pavia, Italy, [6] Neuro-Oncology Unit, IRCCS Mondino Foundation, Pavia, Italy

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons. Although its etiology is still unknown, many genes have been found to be implicated in ALS pathogenesis. The Cu/Zn superoxide dismutase (*SOD1*) gene was the first to be identified. Currently, more than 230 mutations in the *SOD1* gene have been reported. p.D90A (p. Asp90Ala) is the most common *SOD1* mutation worldwide. It shows both autosomal and recessive inheritance in different populations. To date, five Italian patients with the heterozygous p.D90A mutation have been reported. None of them complained of laryngological symptoms as the initial manifestation of ALS, although they had atypical clinical features. We describe a long-survival patient carrying heterozygous p.D90A mutation who presented with severe laryngospasm due to bilateral vocal cord paralysis. We suggest that genetic analysis may help to diagnose ALS with insidious onset like hoarseness, laryngospasm, and other type of voice disturbances.

Keywords: Amyotrophic Lateral Sclerosis, *SOD1*, p.D90A, laryngospasm, case report

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Wladimir Bocca Vieira De Rezende Pinto,
Federal University of São Paulo, Brazil

***Correspondence:**
Cristina Cereda
cristina.cereda@mondino.it

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INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder that affects the upper and lower motor neurons in the spinal cord, brainstem, and motor cortex (1). Its etiology is still unknown, but it is now accepted to be based on a complex interplay between environmental factors and genetic background (1). Most cases of ALS are sporadic (SALS), while about 10% of cases are familial (FALS), even though SALS and FALS are clinically identical and have both a genetic basis (1). In 1993, Rosen et al. discovered the first ALS-associated gene, *SOD1*, that is located on chromosome 21q22.11 and encodes a Cu/Zn-binding superoxide dismutase (2–4). To date, more than 230 mutations have been reported to be ALS-associated, but it is still controversial whatever all of them are disease-causative (3, 4).

Currently, p.D90A (p.Asp90Ala) is the most common *SOD1* mutation (3, 4) and is inherited as both a recessive and dominant trait in different populations (5). In northern Scandinavia, p.D90A heterozygous carriers are unaffected, while they developed ALS in Belgium (5).

In Italy, five patients carrying the heterozygous p.D90A mutation have been reported (6–9).

We herein report the heterozygous p.D90A mutation in a long-survival patient with ALS and laryngospasm as the initial manifestation.

CASE PRESENTATION

A 58-year-old male was admitted to our department in May 2016 because of a 12-year history of progressive muscle weakness in the upper limbs. Family history for neurodegenerative diseases was negative. He was diagnosed with Gilbert syndrome and mild tricuspid valve regurgitation and he suffered from tachyarrhythmia and dyspepsia due to esophagitis. He presented with hypophonia due to a bilateral vocal fold paralysis occurred in 1999. Electromyography (EMG) showed a bilateral axonal neuropathy involving the superior and recurrent laryngeal nerves, especially on the left. As laryngeal spasms caused episodes of respiratory failure, he underwent a left laser posterior cordotomy. He began to suffer from low back pain after an accidental fall due to a disc herniation at L5-S1. Furthermore, mild postural tremor affecting the hands appeared and neurological examination showed muscle weakness in the proximal segments of the upper limbs. He was initially diagnosed with spinobulbar atrophy. Genetic tests for Kennedy's disease were negative. Furthermore, Brown-Vialetto-Van Laere syndrome was ruled out ex juvantibus by administering high-dose riboflavin and then by molecular analysis.

At first neurological examination, he had bilateral muscle weakness and hypotrophy of the proximal segments of the upper limbs, especially of the deltoids (Medical Research Council, MRC 3+ on the right side, 4− on the left side), of the both biceps brachii (MRC 3+) and triceps brachii (3−). Muscle strength was preserved in the distal segments of the arms. Triceps reflex was absent, while the other tendon reflexes of the upper limbs were normal. Lower limbs show no pathological signs except bilaterally decreased ankle jerk reflexes. Flexor plantar responses were assessed bilaterally. No fasciculations were detected. Cranial nerves were normal and there are no signs of sensory impairment. Magnetic Resonance Imaging (MRI) of the chest and cervical spine showed bilateral changes in the muscles of the rotator cuff, especially on the right, depending on a chronic neurogenic damage, and degeneration of the inferior roots of the brachial plexus. EMG and electroneuronography (ENoG) revealed diffuse motor axonopathy in spinal and bulbar regions with fasciculation potentials and denervation activity at rest, especially in the right deltoid, in the left thyroarytenoid muscle and in the thoracic paraspinal muscles on both sides. Sensory evoked potentials (SEPs) were normal. Routine laboratory investigations showed high levels of serum creatine

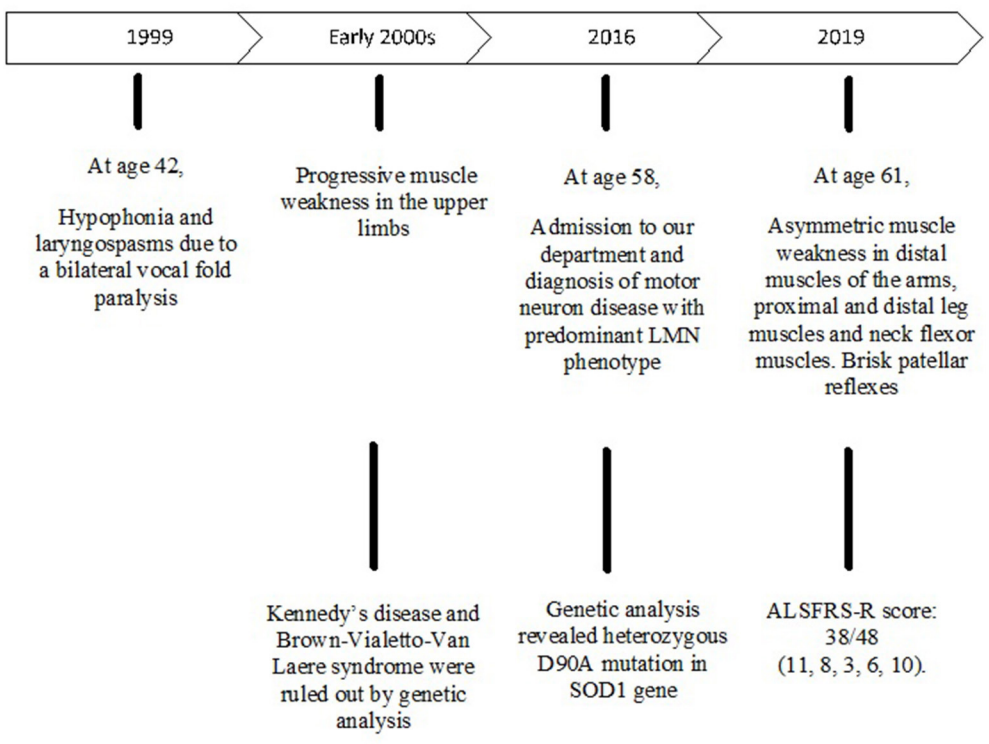

FIGURE 1 | Sanger sequencing electropherogram; "*" shows the variant c.272A>C in the exon 4 of *SOD1* in the patient.

kinase (359 UI/l; n.v.: 39–308). A diagnosis of motor neuron disease with predominant lower motor neuron phenotype was made.

The patient's disease progression was monitored at 3-month intervals by testing muscle strength and performing ALSFRS-R (ALS-Functional Rating Scale Revised). Disease course was slowly progressive and he was clinically stable (**Figure 1**).

After a 3-year follow up, clinical examination showed weakness of neck flexor muscles (MRC 4), of proximal segments of the upper limbs (MRC 3–4), of the opponens pollicis muscle bilaterally (MRC 4), of the left iliopsoas, of the left peroneal muscles and of the left plantar flexors muscles (MRC 4, 5). Right plantar response was indifferent, while patellar reflexes spread to adductor muscles of the thigh. He complained of dysphagia and weight loss. According to the revised El Escorial criteria, a diagnosis of probable ALS-laboratory supported was made. The ALSFRS-R score was 38/48 (4, 7, 9–11).

GENETIC ANALYSIS

After obtaining a written informed consent, Next Generation Sequencing (NGS) analysis was performed, using a customized panel of 174 genes related to neurodegenerative diseases as described in the **Supplementary Material**. We identified the heterozygous variant g.12669A>C, c.272A>C in the exon 4 of the *SOD1* gene resulting in the amino acid change p.Asp90Ala (**Figure 2**). The c.272A>C mutation was then confirmed by Sanger sequencing.

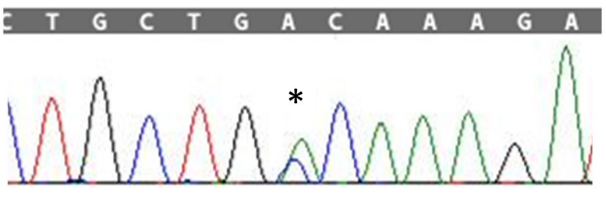

FIGURE 2 | Timeline with relevant data from the episode of care.

DISCUSSION

In this study we described an ALS patient presenting with laryngospasm as disease onset and a predominant lower motor neuron phenotype involving the proximal segments of the upper limbs. The clinical course was slowly progressive, according to previous evidence of longer survival in patients with a lower motor neuron phenotype (12).

Genetic analysis showed a heterozygous p.D90A mutation of the *SOD1* gene.

To date, five Italian patients with the heterozygous p.D90A have been reported (6–9) (**Table 1**). They had a spinal onset involving lower and/or upper limbs typically with muscle weakness at an average age of 46, 6 years. Neurological examination showed both upper and lower motor neuron signs. Two patients complained of sensory disturbances. The disease onset did not show vocal cord paralysis in any patient except our case (6–9). Although this variant is not associated with a distinct phenotype, Origone et al. concluded that patients carrying the heterozygous p.D90A mutation have atypical clinical features. So, they suggested to perform molecular analysis looking for *SOD1* mutations in both SALS and FALS patients with atypical disease onset (8).

Although voice disturbances are rare, they are an insidious type of ALS onset (11).

Laryngological symptoms included: hoarseness, dysphonia, hypophonia/aphonia, non-productive cough, and life-threatening conditions as inspiratory stridor and laryngospasm.

Chen and Garrett demonstrated that a significant number of ALS patients with bulbar onset are initially referred to otolaryngologists. One thousand seven hundred fifty-nine patients were evaluated at a voice center from 1998 to 2003: <1% later received a diagnosis of ALS. In contrast, 20% of 220 ALS patients seen at the neurological clinic had bulbar onset and about half of them complained of dysphonia. When

TABLE 1 | Clinical features of ALS patients carrying the heterozygous p.D90A-*SOD1* mutation in the Italian population.

	Sex	Familiarity	Age of onset (years)	Site of onset	Type of onset	Early sensory symptoms	Bulbar involvement
Battistini et al. (6)	M	Yes	42	Spinal (lower limbs)	Sensory-motor neuropathy	Yes	No
Luigetti et al. (9)	M	No	45	Spinal (lower limbs)	Fasciculations Gait impairment	No	Pseudobulbar sign
Origone et al. (8)	M	No	41	Spinal (lower limbs)	Weakness Gait impairment	No	Spastic dysarthria Moderate dysphagia
Giannini et al. (7)	M	No	58	Spinal	Weakness and atrophy of the left hand Leg cramps	No	No
Giannini et al. (7)	F	No	47	Spinal	Foot drop	Yes	Dysarthria Dysphagia

M, male; F, female.

dysphonia was found, patients were initially referred to an otolaryngologist rather than a neurologist. Unfortunately, they were often misdiagnosed due to previous misleading diagnoses, dysarthria mistaken for dysphonia, subtle symptoms, and signs of neuromuscular disease overlooked by physicians. Therefore, the authors concluded that ALS diagnosis is a significative challenge in the otolaryngology practice (11).

Vocal cord problems may present with acute dyspnoea due to glottic narrowing or even glottic occlusion (10). In ALS, glottic narrowing has been supposed to depend on two types of vocal cord dysfunction: the supranuclear non-paralytic type causing an overactivity of vocal cord adductors and/or the infranuclear paralytic type consisting in neurogenic atrophy and weakness of the posterior cricoarytenoid muscle (10). Glottic narrowing is clinically symptomatic, resulting in inspiratory stridor, especially at an early disease stage in patients with a good vital capacity (10).

Laryngospasm is defined as a rapid and involuntary closure of the larynx (10). It is well-known that it may occur during intubation/extubation procedures and during disease progression probably due to a combination of gastro-esophageal reflux disease (GORD), aspiration of gastric contents, and dysphagia (10, 19). Diet modifications and pharmacological therapy with prokinetic agents and proton pump inhibitors (PPI) are recommended (19).

Instrumental assessment consists in laryngoscopy and laryngeal electromyography (10).

Changing to an upright position of the trunk, stabilizing the body through the fixation of the arms and slowly breathing are sufficient maneuvers to shorten the spasm episode (19).

Treatment of life-threatening vocal cord disturbances ranges from intubation and cricothyroidotomy to tracheotomy according to patient's advance directives (10).

Among neuromuscular diseases, laryngospasm is common at an early stage of Kennedy disease, while it is essentially a rare type of ALS onset (20).

In addition to our patient, literature reported other cases of ALS patients experiencing laryngospasm as initial manifestation of ALS (10, 11, 20).

It should be emphasized that vocal cord dysfunction occurs also in ALS patients without major bulbar involvement, as in the case of our patient (10, 21).

SALS and FALS patients presenting with laryngological onset has been reported to carry a missense mutation in the *SOD1* gene, as shown in **Table 2** (13–18).

Hermann et al. described a patient with hoarseness and muscle weakness in the proximal segments of the upper limbs at onset. As our patient, he had a bilateral compromission of vocal folds and a predominant lower motor neuron phenotype although upper motor neuron signs were present too. However, the disease progression was different from our case and the patient died 15 months after disease onset. Genetic analysis revealed a heterozygous missense mutation c.337 A>T in exon 4 of the *SOD1* gene. Authors suggested a pathogenic role for this mutation (17).

TABLE 2 | Previously reported ALS patients carrying missense *SOD1* mutations and presenting with voice disturbance.

Variant in protein/reference	p.I149T Fukae et al. (13)	p.D101Y Tan et al. (14)	p.A4V Salameh et al. (15)	p.I151T Kostrzewa et al. (16)	p.I113F Hermann et al. (17)	p.G147S Origone et al. (18)
Familial/Sporadic ALS	FALS	FALS	FALS	FALS	FALS	SALS
Sex	F	M	M	F	M	M
Age at onset (yy)	43	57	73	48	49	56
Type of laryngological onset	Hoarseness due to bilateral vocal cord paralysis	Hoarseness due to bilateral vocal cord paralysis	Slurred voice	Dysarthria and dysphagia	Hoarseness due to bilateral vocal cord paralysis	Episodic dyspnoea and hoarseness due to bilateral vocal cord paralysis (right vocal cord at first)
Clinical features	Severe bulbar palsy, diffuse fasciculations, brisk deep tendon reflex, preserved limbs muscle strength	Dysphagia, progressive muscle weakness, and wasting in the right upper arm and on the right side of the face and tongue, atrophy of both scapular regions and the precordium, normal deep tendon reflexes	Right vocal cord paralysis, facial diplegia, dysarthria, dyspnoea, dysphagia, proximal right arm and mild proximal right leg weakness, arms fasciculations, normal/hyporeflexic deep tendon reflexes	Asymmetrical tetraparesis with muscle atrophy and fasciculations, brisk deep tendon reflexes	Muscle weakness in the proximal segments of the upper limbs, fasciculations of the tongue and shoulder muscles, brisk deep tendon reflexes	Progressive dyspnoea, inspiratory stridor, muscle weakness in four limbs, atrophy of small hand muscles, diffuse fasciculations, dysphagia, normal deep tendon reflexes
Progression	Tracheostomy 17 months after disease onset	Death 11 months after disease onset due to respiratory failure	Death 14 months after disease onset	Severe bulbar palsy and respiratory weakness	Death 15 months after disease onset	Death 8 months after disease onset due to respiratory failure

FALS, Familiar Amyotrophic Lateral Sclerosis; SALS, Sporadic Amyotrophic Lateral Sclerosis; yy, years; M, male; F, female.

Table 2 summarizes other patients affected by motor neuron disease with voice disturbances as disease onset. Most of them presented with hoarseness and had a rapid progressive disease course and none of them carried the same mutation as our case. Some of them showed a predominant lower motor neuron phenotype (14, 15, 18).

CONCLUSION

ALS onset may be insidious and subtle including laryngological symptoms like hoarseness, dysphonia, inspiratory stridor, and laryngospasm. Therefore, many ALS patients are initially referred to an otolaryngologist rather than a neurologist. Although the role of genetic testing is still controversial in clinical practice, our findings suggest that molecular analysis of the *SOD1* gene may be indicated in ALS patients with laryngological onset.

DATA AVAILABILITY STATEMENT

Some of the original contributions presented in the study are publicly available. This data can be found here: ZENODO: https://zenodo.org/record/5361094. The rest of the data can be provided by request to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

GC, LD, EA, and MC contributed to writing of the manuscript and to critical revision of the manuscript. CC and IP contributed to genetic analysis. All authors gave important contributions to the final form of the manuscript.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Pasinelli P, Brown RH. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. *Nat Rev Neurosci.* (2006) 7:710–23. doi: 10.1038/nrn1971
2. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D. et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* (1993) 362:59–62. doi: 10.1038/362059a0.
3. Mathis S, Goizet C, Soulages A, Vallat J-M, Masson GL. Genetics of amyotrophic lateral sclerosis: a review. *J Neurol Sci.* (2019) 399:217–26. doi: 10.1016/j.jns.2019.02.030
4. Diamanti L, Gagliardi S, Cereda C, Ceroni M. Genetics of ALS and correlations between genotype and phenotype in ALS — a focus on italian population. In: *Current Advances in Amyotrophic Lateral Sclerosis.* InTech (2013). Available online at: https://www.intechopen.com/books/current-advances-in-amyotrophic-lateral-sclerosis/genetics-of-als-and-correlations-between-genotype-and-phenotype-in-als-a-focus-on-italian-population (accessed August 25, 2019). doi: 10.5772/56547
5. Robberecht W, Aguirre T, Van Den Bosch L, Tilkin P, Cassiman JJ, Matthijs G. D90A heterozygosity in the SOD1 gene is associated with familial and apparently sporadic amyotrophic lateral sclerosis. *Neurology.* (1996) 47:1336–9. doi: 10.1212/WNL.47.5.1336
6. Battistini S, Giannini F, Greco G, Bibbò G, Ferrera L, Marini V, et al. *SOD1* mutations in amyotrophic lateral sclerosis. *J Neurol.* (2005) 252:782–8. doi: 10.1007/s00415-005-0742-y
7. Giannini F, Battistini S, Mancuso M, Greco G, Ricci C, Volpi N, et al. D90A-SOD1 mutation in ALS: the first report of heterozygous Italian patients and unusual findings. *Amyotroph Lateral Scler.* (2010) 11:216–9. doi: 10.3109/17482960902721642
8. Origone P, Caponnetto C, Mascolo M, Mandich P. Heterozygous D90A-SOD1 mutation in an Italian ALS patient with atypical presentation. *Amyotroph Lateral Scler.* (2009) 10:492–492. doi: 10.3109/17482960903055966
9. Luigetti M, Conte A, Madia F, Marangi G, Zollino M, Mancuso I, et al. Heterozygous SOD1 D90A mutation presenting as slowly progressive predominant upper motor neuron amyotrophic lateral sclerosis. *Neurol Sci.* (2009) 30:517–20. doi: 10.1007/s10072-009-0125-8
10. van der Graaff MM, Grolman W, Westermann EJ, Boogaardt HC, Koelman H, van der Kooi AJ, et al. Vocal cord dysfunction in amyotrophic lateral sclerosis. *Arch Neurol.* (2009) 66:1329–33. doi: 10.1001/archneurol.2009.250
11. Chen A, Garrett CG. Otolaryngologic presentations of amyotrophic lateral sclerosis. *Otolaryngol Neck Surg.* (2005) 132:500–4. doi: 10.1016/j.otohns.2004.09.092
12. Schito P, Ceccardi G, Calvo A, Falzone YM, Moglia C, Lunetta C, et al. Clinical features and outcomes of the flail arm and flail leg and pure lower motor neuron MND variants: a multicentre Italian study. *J Neurol Neurosurg Psychiatry.* (2020) 91:1001–3. doi: 10.1136/jnnp-2020-323542
13. Fukae J, Kubo S, Hattori N, Komatsu K, Kato M, Aoki M, et al. Hoarseness due to bilateral vocal cord paralysis as an initial manifestation of familial amyotrophic lateral sclerosis. *Amyotroph Lateral Scler.* (2005) 6:122–4. doi: 10.1080/14660820510034451
14. Tan C-F, Piao Y-S, Hayashi S, Obata H, Umeda Y, Sato M, et al. Familial amyotrophic lateral sclerosis with bulbar onset and a novel Asp101Tyr Cu/Zn superoxide dismutase gene mutation. *Acta Neuropathol.* (2004) 108:332–6. doi: 10.1007/s00401-004-0893-4
15. Salameh JS, Atassi N, David WS. SOD1 (A4V)-mediated ALS presenting with lower motor neuron facial diplegia and unilateral vocal cord paralysis. *Muscle Nerve.* (2009) 40:880–2. doi: 10.1002/mus.21321
16. Kostrzewa M, Damian MS, Müller U. Superoxide dismutase 1: identification of a novel mutation in a case of familial amyotrophic lateral sclerosis. *Hum Genet.* (1996) 98:48–50. doi: 10.1007/s00439005015 7
17. Hermann A, Reuner U, Ziethe G, Bräuer A, Gölnitz U, Rolfs A, et al. Vocal cord paralysis and rapid progressive motor

neuron disease by the I113F mutation in SOD1 gene. *Amyotroph Lateral Scler.* (2011) 12:382–4. doi: 10.3109/17482968.2011.565775

18. Origone P, Caponnetto C, Mantero V, Cichero E, Fossa P, Geroldi A, et al. Fast course ALS presenting with vocal cord paralysis: clinical features, bioinformatic and modelling analysis of the novel SOD1 Gly147Ser mutation. *Amyotroph Lateral Scler.* (2012) 13:144–8. doi: 10.3109/17482968.2011.614254
19. Kühnlein P, Gdynia HJ, Sperfeld AD, Lindner-Pfleghar B, Ludolph AC, Prosiegel M, et al. Diagnosis and treatment of bulbar symptoms in amyotrophic lateral sclerosis. *Nat Clin Pract Neurol.* (2008) 4:366–74. doi: 10.1038/ncpneuro0853
20. Sperfeld A-D, Hanemann CO, Ludolph AC, Kassubek J. Laryngospasm: an underdiagnosed symptom of X-linked spinobulbar muscular atrophy. *Neurology.* (2005) 64:753–4. doi: 10.1212/01.WNL.0000151978.74467.E7
21. Tsunoda K, Takazawa M, Chong T, Morita Y. Slow, slurred speech as an initial complaint in amyotrophic lateral sclerosis. *Auris Nasus Larynx.* (2019) 46:193–5. doi: 10.1016/j.anl.2018.07.007

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CASE REPORT
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Facial Paresthesia, a Rare Manifestation of Hereditary Neuropathy With Liability to Pressure Palsies: A Case Report

Lisa De Kock[1], Fréderic Van der Cruyssen[2], Leonore Gruijthuijsen[1] and Constantinus Politis[2]*

[1] Faculty of Medicine, University Leuven and Maxillofacial Surgery Department, University Hospitals Leuven, Leuven, Belgium, [2] OMFS IMPATH Research Group, Department of Imaging and Pathology, Faculty of Medicine, University of Leuven and Oral and Maxillofacial Surgery, University Hospitals Leuven, Leuven, Belgium

Trigeminal sensory neuropathy can be caused by a variety of conditions, including local, traumatic, iatrogenic, or systemic causes. Diagnosis and management remain a challenge for maxillofacial surgeons and neurologists. Therefore, a good clinical examination and objective tests and imaging are needed when diagnosing patients who present with facial numbness. We present a case with spontaneous episodes of facial paresthesia. He was diagnosed with hereditary neuropathy with liability to pressure palsies (HNPP), a rare condition that affects the peripheral nerves. Only a few case reports that describe involvement of the cranial nerves in patients with HNPP were found in the literature, and facial paresthesia has not been previously reported.

Keywords: paresthesia, hereditary neuropathy with liability to pressure palsy, trigeminal neuropathy, PMP22 deletion, case report

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****Correspondence:***
Lisa De Kock
lisadekock91@gmail.com

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INTRODUCTION

According to the International Association for the study of Pain (IASP) terminology (1), paresthesia is defined as an abnormal sensation, whether spontaneous or provoked. It is not considered unpleasant, in contrast to dysesthesia, which is preferentially used to describe an abnormal sensation that is considered unpleasant. Paresthesia is frequently encountered in clinical practice by maxillofacial surgeons and neurologists, though diagnosis and management can be challenging. The condition has a broad differential diagnosis, which often makes it difficult to find the cause. Local, traumatic, or iatrogenic factors are the most common causes of paresthesia in the maxillofacial region, and systemic causes are rare (2). We report the case of a patient who presented with spontaneous episodes of facial paresthesia and was diagnosed with hereditary neuropathy with liability to pressure palsies (HNPP). The involvement of cranial nerves has seldom been described in case studies of HNPP. The purpose of this case report is to review this rare presentation and to examine the process for differential diagnosis in a patient with spontaneous paresthesia in the distribution area of the trigeminal nerve.

CASE DESCRIPTION

A 25-year-old Afro-American male was referred to the Department of Oral and Maxillofacial Surgery by his family doctor because of recurrent episodes of facial paresthesia. These episodes

TABLE 1 | Quantitative sensory testing according to the DFNS protocol.

Variable		
VAS pain score	0/10	
Allodynia	–	
Hyperpathia	–	
Hyperalgesia	–	
Hypoesthesia	–	
Paresthesia	+	
Affected dermatome, %	0	
Directional sense	10/10	
Stimulus localization	5/5	
Subjective score	10/10	
	Left	**Right**
Mechanical pain threshold (Von Frey filaments), mN	0.08	0.08
Tactile threshold, mN	64	64
Two-point discrimination, mm	5	5
Temperature testing		
Cold detection threshold, °C	32.1	31.5
Warm detection threshold,°C	44.6	39.8
Cold pain threshold, °C	0	4.2
Heat pain threshold, °C	44.4	43.5

VAS, visual analogue scale; mN, Millinewton.

spontaneously started 3 months prior and were observed for the first time after severe alcohol consumption. They could develop any time during the day, but mostly occurred during meals and long conversations. The paresthesia was reported bilaterally in the distribution area of the mandibular nerve, particularly the mental and auriculotemporal nerve. No pain or hypoesthesia was described. He did not have recent dental treatment. These complaints impeded his daily life and professional activities due to concentration problems. A few weeks earlier, he experienced hypoesthesia in the left little finger, left foot, and the medial side of the left wrist for approximately 1 week. These symptoms were often encountered in the past, but never lasted as long as 1 week. The family doctor prescribed corticosteroids, but this did not improve the symptoms.

The patient's medical history included heterozygote sickle cell trait (HbAS) and alpha-thalassemia minor, for which he was scheduled for regular routine follow-ups with his hematologist. He had a history of smoking (2 pack-years). He had experienced tinnitus of unknown etiology for more than 4 years. In addition, the patient followed up regularly with a cardiologist for a second-degree atrioventricular block type 1. At 21 years old, he was diagnosed with HNPP. Genetic analysis of *PMP22* confirmed deletion of the chromosome 17p11.2 region. Electromyography (EMG) of the upper and lower limbs showed multifocal demyelinating anomalies with diminished sensory and motor conduction velocity. In this regard, he occasionally encountered transient episodes of muscle weakness and hypoesthesia in the arms and legs, as mentioned earlier.

Upon clinical examination, the trigeminal and facial nerves were normal. Symptoms could not be elicited. There were no clinical signs of a disorder in the temporo-mandibular joint, and further clinical examination was normal. A panoramic radiograph and lateral head film showed no aberrancies except horizontally impacted wisdom teeth. Blood analysis showed microcytic hypochromic anemia, which can be attributed to the alpha-thalassemia minor. He had no vitamin deficiencies. HIV infection, hepatitis B, and syphilis were excluded by appropriate tests. Before our exam, he had been assessed by the neurology department. This showed a symmetric motor function of the facial nerve, normal sensibility of the trigeminal nerve and a normal examination of the remaining cranial nerves. Strength testing revealed a reduced flexion strength of the left little finger and reduced extension strength of the left great toe. On the left side the little finger and medial side of the hand as well as the upper side of the left foot showed a diminished light touch and pinprick sensibility. Vibration perception of the left hand and foot was normal. The right side was completely unremarkable. Coordination of a heel-shin slide and finger-to-toe test was normal. The reflexes were symmetric. An EMG of the left ulnar and median nerve did not yield pathological findings other than the known HNPP. Blink testing of the facial muscles were within normal limits. Quantitative sensory testing (QST) according to the DFNS protocol (3) revealed thermal sensory disturbances in the trigeminal distribution. On the right side, the cold pain threshold was increased, and the warmth detection threshold was clearly lower (**Table 1**). Magnetic resonance neurography (MRN) of the trigeminal nerve showed slightly increased nerve calibers and signal intensities, more pronounced on the right inferior alveolar nerve (IAN) (**Figure 1**). Intramuscular injections with vitamin B complex once a week were prescribed. After 6 weeks the patient reported improvement of his symptoms. The episodes of facial paresthesia were decreased in number and intensity and did no longer impeded his daily life activities. Vitamin B supplements were interrupted without recurrence or exacerbation of symptoms.

DISCUSSION

Finding the cause of trigeminal sensory neuropathy can be a diagnostic challenge because it can be caused by a variety of disorders. The main causes of paresthesia in the maxillofacial region are dental in origin (2), whereas other systemic causes include demyelinating diseases, connective tissue diseases, systemic infection, and primary or metastatic malignancies, and can even be the first manifestation of multiple sclerosis (5). Forty-eight percent of dental causes have been attributed to a dental procedure and involve the IAN and lingual nerves (2). In this case, dental causes were excluded by clinical and radiological examination. A differential diagnosis was made between sickle cell disease (SCD), thalassemia minor, and HNPP as the possible cause of the idiopathic bilateral facial paresthesia. Other neurological causes were excluded by the Department of Neurology.

SCD is a group of hematological disorders that cause sickle-shaped erythrocytes to disrupt the blood flow in small vessels (6). This can lead to an acute vaso-occlusive sickle-cell crisis,

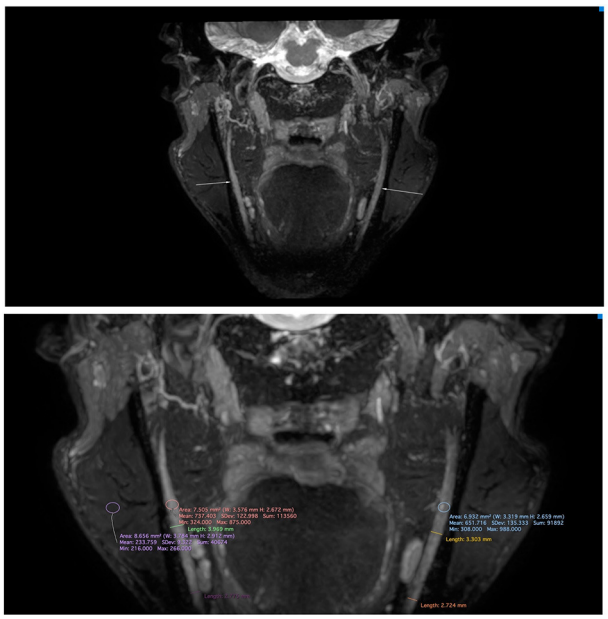

FIGURE 1 | Magnetic resonance neurography of the mandibular nerve on a 3T Philips Ingenia with standard 32 channel head coil (Philips, Best, the Netherlands) applying a 3D CRANI sequence (4). The image was reconstructed using maximum intensity projection and multiplanar reformatting. Note the slightly increased nerve calibers and signal intensities, which are more pronounced on the right inferior alveolar nerve.

characterized by tissue ischemia, inflammation, and periodic episodes of pain. Clinically evident peripheral nervous system involvement is uncommon in SCD patients. However, subclinical peripheral nerve association has been described (7). Gadallah et al. (8) also concluded that trigeminal neuropathy may be associated with SCD, either subclinically or symptomatically. Few case reports (9, 10) have described a relationship between SCDs and mental nerve neuropathy. Most reported cases had a homozygous (HbSS) or compound heterozygous (HbSC) form of the disease. The patients presented with bone pain crises at the mandible, followed by a burning sensation and numbness of the lower lip. It is thought that a vaso-occlusive sickle cell crisis may cause painful neuropathy due to nerve ischemia, infarction, or from compression as a result of bone infarct or osteomyelitis of the mandible (8). The patient in this case report was known to have the sickle cell trait, which is strictly not a form of SCD (6). He did not encounter the bone pain crises that often precede paresthesia or numbness. Thus, SCD was excluded as a cause of his symptoms.

Alpha-thalassemia is an inherited hemoglobinopathy characterized by impaired synthesis of alpha-globin chains, leading to excess beta-globin chains (11). This deficient production most frequently results from a deletion of one or more alleles (HBA1 and HBA2). The clinical presentation can vary depending on the number of affected alleles. Thus, alpha-thalassemia can be classified into different subtypes: silent alpha-thalassemia, alpha-thalassemia minor (or alpha-thalassemia trait), hemoglobin H disease (HbH), and hemoglobin Bart's hydrops fetalis. Alpha-thalassemia minor, the subtype in this case, is caused by a two-gene deletion, one in each chromosome, which causes microcytosis and hypochromia with absent or mild anemia, generally with no other symptoms. To the best of our knowledge, there are no records describing a correlation between alpha-thalassemia and peripheral neuropathy. In contrast to alpha-thalassemia, limited studies have (12, 13) reported an association between beta-thalassemia and sensory axonal polyneuropathy affecting the distal segments of the nerves. This phenomenon seems to increase with age, possibly be due to chronic anemia (13). Other hypothetical causes of this neuropathy are iron overload, neurotoxicity of the drug desferriosamine, and bone marrow expansion (14). The patient in this case did not present with any of the aforementioned hypothetical causes of neuropathy in beta-thalassemia. He was diagnosed with alpha-thalassemia minor, which has no proven association with peripheral neuropathy. Therefore, alpha-thalassemia minor was ruled out as a cause of the symptoms.

HNPP is an autosomal dominant disorder that affects the peripheral nerves and is characterized by recurrent episodes of transient mononeuropathy, usually provoked by minor trauma (15). It has a prevalence of 7–16 per 100,000 individuals and is frequently underdiagnosed due to the heterogeneity of the clinical and electrophysiological presentation (16). HNPP is caused genetically by a 1.5 Mb heterozygous deletion of the chromosome 17p11.2-p12 region, including the peripheral myelin protein-22 gene in the majority of cases. Symptoms usually start during adolescence or young adulthood and include episodes of numbness, paresthesia, muscle weakness, and atrophy. The neuropathological presentation includes segmental de- and re-myelination, tomaculous or sausage-like formations with typical segmental thickening of myelin, and diminished conduction velocity and amplitude of the potentials along motor and sensory nerves (17, 18). Electrodiagnostic, histopathological, and genetic testing are essential in diagnosing HNPP (15). The most frequently affected nerves are the median, ulnar, radial, and peroneal nerves, or the brachial plexus at sites commonly exposed to trauma or entrapment due to their anatomical locations (17). Cranial nerves are usually not involved. Only a few case reports (19–24) have described the involvement of the facial, hypoglossal, and other cranial nerves (**Table 2**).

Swallowing dysfunction and vocal cord paralysis have been described in HNPP in relation to hypoglossal neuropathy or recurrent laryngeal nerve palsy (21–23). Recurrent facial palsy was described as a first clinical manifestation in a family diagnosed with HNPP (19). The anatomy of the facial nerve leads to physiological entrapment sites, particularly in its intra-temporal portion, and makes it vulnerable to pressure palsies (20). To the best of our knowledge, facial paresthesia in HNPP has not been reported previously. These patients are predisposed to nerve demyelination, causing the slightest pressure, stretch, or repetitive movement to induce neurological impingement. This patient was previously diagnosed with HNPP, which was confirmed by genetic screening. He sometimes experienced

TABLE 2 | Case reports in the literature describing cranial nerve involvement in patients with HNPP.

Study	Sex	Age (years)	Family history	Other/previous episodes	Cranial nerve involvement	Symptoms	Causative incident	Confirmed by molecular genetic analysis	Follow-up
Corwin and Girardet (21)	Male	74	None	4 episodes of right foot drop following minor lower extremity trauma	Hypoglossal nerve	Dysarthria, inability to protrude or move the tongue laterally, left tongue fasciculations	Bilateral carotid endarterectomy	Yes	5 years: remaining sensation of swelling of the left tongue, atrophy of the right tongue, curling of the tongue tip to the left
Poloni et al. (19)	Male	16	Father diagnosed with HNPP	Left inferior peripheral facial palsy, complete right facial palsy, left peroneal deficit, transient right radial deficit	Facial nerve	Facial palsy	/	Yes	All episodes recovered in a few weeks
Ohkoshi et al. (23)	Female	19	Father and brother diagnosed with HNPP	2 episodes of hand drop, with bilateral severe weakness of wrist extensor muscles and finger extensor muscles and mild paresthesia on the radial side of both hands and forearms	Recurrent laryngeal nerve	Aphonia and hoarseness, dysphagia, aspiration of food and drink into the trachea	Sleeping in the prone position the night before	Yes	Complete recovery after 6 weeks, both hand drops remained
Iwasaki et al. (20)	Female	40	None	5-year history of increasing weakness in the lower extremities and deteriorating gait due to sensation of heaviness in her legs	CNS (trigeminal, facial, and hypoglossal nerves)	Dysesthesia in the left forehead, weakness of bilateral facial muscles, no protrusion of the tongue possible	/	Yes	/
Winter and Juel (22)	Female	19	Mother diagnosed with Charcot-Marie-Tooth disease	None	Hypoglossal nerve	Dysphagia, lingual dysarthria, tongue weakness, tongue deviation to the right with protrusion	Sleeping in a seated position, supporting the head with her right palm	Yes	Complete recovery in 10 days
Lorenzoni et al. (24)	Male	41	Sister and nephew similar symptoms, no proven HNPP	Recurrent episodes of mononeuropathies affecting mainly the ulnar, radial and peroneal nerves followed by spontaneous improvement since the age of 12 years	Recurrent laryngeal nerve, hypoglossal nerve	Swallowing dysfunction	None	Yes	Spontaneous improvement after few weeks, but with residual dysfunction when drinking liquid

recurrent episodes of muscle weakness and hypoesthesia in the arms and legs. As episodes of paresthesia are a symptom of HNPP and it is a slow progressive disorder, this facial paresthesia could be part of further progression of the disease. In this case, chewing and long conversations, which are repeated movements, could be a causative factor. Furthermore, QST and MRN showed changes in the trigeminal nerve, which could indicate a small fiber neuropathy. Currently, no treatment is available for HNPP (15). Patients usually recover from their symptoms, though it can sometimes take several months. Current management focuses mainly on preventing damage to the peripheral nerves. Activities that may provoke pressure palsies, such as prolonged sitting with crossed legs, repetitive movements of the wrist, and prolonged leaning on elbows, should be avoided.

CONCLUSION

Clinical diagnosis and management of patients with spontaneous facial paresthesia remains a challenge for maxillofacial surgeons. Etiology can differ, but HNPP has never been reported among the known possible causes. After exclusion of local or iatrogenic etiologies, systemic or neurological causes should be considered. A good clinical examination and objective tests and imaging are imperative in obtaining an accurate diagnosis. Even though the presentation is rare, physicians should consider HNPP in the differential diagnosis of transient idiopathic trigeminal mononeuropathy, especially in the context of recurrent pressure palsies, distal extremity weakness, reduced tendon reflexes, or family history of hereditary neuropathy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LD wrote the manuscript with contributions of FV and LG. FV treated the patient. CP supervised the project. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. International Association for the Study of Pain. *IASP Terminology*. (2017). Available online at: https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698#Paresthesia (accessed March 14, 2021).
2. Shadmehr E, Shekarchizade N. Endodontic periapical lesion-induced mental nerve paresthesia. *Dent Res J.* (2015) 12:192–6.
3. Rolke R, Baron R, Maier C, Tölle TR, Treede R-D, Beyer A, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. *Pain.* (2006) 123:231–43. doi: 10.1016/j.pain.2006.01.041
4. Van der Cruyssen F, Croonenborghs TM, Renton T, Hermans R, Politis C, Jacobs R, et al. Magnetic resonance neurography of the head and neck: state of the art, anatomy, pathology and future perspectives. *Br J Radiol.* (2021) 94:20200798. doi: 10.1259/bjr.20200798
5. Genc Sen O, Kaplan V. Temporary mental nerve paresthesia originating from periapical infection. *Case Rep Dent.* (2015) 2015:457645. doi: 10.1155/2015/457645
6. Ware RE, de Montalembert M, Tshilolo L. Sickle-cell disease. *Lancet.* (2017) 390:311–23. doi: 10.1016/S0140-6736(17)30193-9
7. Okuyucu EE, Turhanoglu A, Duman T, Kaya H, Melek IM, Yilmazer S. Peripheral nervous system involvement in patients with sickle cell disease. *Eur J Neurol.* (2009) 16:814–8. doi: 10.1111/j.1468-1331.2009.02592.x
8. Gadallah N, El Hefnawy H, Ahmed S, Ali J. Trigeminal nerve electrophysiological assessment in sickle cell anemia: correlation with disease severity and radiological findings. *Egypt Rheumatol Rehabil.* (2015) 42:73–9. doi: 10.4103/1110-161X.157865
9. Konotey-Ahulu FI. Mental-nerve neuropathy: a complication of sickle-cell crisis. *Lancet.* (1972) 2:388. doi: 10.1016/S0140-6736(72)91788-6
10. Friedlander AH, Genser L, Swerdloff M. Mental nerve neuropathy: a complication of sickle-cell crisis. *Oral Surg Oral Med Oral Pathol.* (1980) 49:15–7. doi: 10.1016/0030-4220(80)90025-0
11. Muncie JH CJ. Alpha and beta-thalassemia. *Am Fam Phys.* (2009) 80:339–44. Available online at: https://www.aafp.org/afp/2009/0815/p339.html
12. Sawaya RA, Zahed L, AT. Peripheral neuropathy in thalassemia. *Ann Saudi Med.* (2006) 26:358–363. doi: 10.5144/0256-4947.2006.358
13. Papanastasiou DA, Papanicolaou D, Magiakou AM, Beratis NG, Tzebelikos E, Papapetropoulos T. Peripheral neuropathy in patients with beta-thalassaemia. *J Neurol Neurosurg Psychiatry*. (1991) 54:997–1000. doi: 10.1136/jnnp.54.11.997
14. Nemtsas P, Arnaoutoglou M, Perifanis V, Koutsouraki E, Orologas A. Neurological complications of beta-thalassemia. *Ann Hematol.* (2015) 94:1261–5. doi: 10.1007/s00277-015-2378-z
15. Attarian S, Fatehi F, Rajabally YA, Pareyson D. Hereditary neuropathy with liability to pressure palsies. *J Neurol.* (2019) 267:2198–206. doi: 10.1007/s00415-019-09319-8
16. van Paassen BW, van der Kooi AJ, van Spaendonck-Zwarts KY, Verhamme C, Baas F, de VM. PMP22 related neuropathies: Charcot-Marie-Tooth disease type 1A and hereditary neuropathy with liability to pressure palsies. *Orphanet J Rare Dis.* (2014) 9:38. doi: 10.1186/1750-1172-9-38
17. Koszewicz M, Martynów R. Hereditary neuropathy with liability to pressure palsies. *Case Rep Clin Pract Rev.* (2002) 3:176–80. Available online at: https://www.amjcaserep.com/download/index/idArt/474513
18. Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, et al. DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell.* (1993) 72:143–51. doi: 10.1016/0092-8674(93)90058-X

19. Poloni TE, Merlo IM, Alfonsi E, Marinou-Aktipi K, Botti S, Arrigo A, et al. Facial nerve is liable to pressure palsy. *Neurology*. (1998) 51:320–2. doi: 10.1212/WNL.51.1.320
20. Iwasaki Y, Iguchi H, Ikeda K, Kano O. CNS involvement in hereditary neuropathy with pressure palsies (HNPP). *Neurology*. (2007) 68:2046. doi: 10.1212/01.wnl.0000268588.67446.3e
21. Corwin HM, Girardet RE. Hereditary neuropathy with liability to pressure palsies mimicking hypoglossal nerve injuries. *Neurology*. (2003) 61:1457–8. doi: 10.1212/01.WNL.0000094207.10032.BA
22. Winter WC, Juel VC. Hypoglossal neuropathy in hereditary neuropathy with liability to pressure palsy. *Neurology*. (2003) 61:1154–5. doi: 10.1212/01.WNL.0000086808.56096.DA
23. Ohkoshi N, Kohno Y, Hayashi A, Wada T, Shoji S. Acute vocal cord paralysis in hereditary neuropathy with liability to pressure palsies. *Neurology*. (2001) 56:1415. doi: 10.1212/WNL.56.10.1415
24. Lorenzoni PJ, Scola RH, Cardoso J, Kay CSK, Fugmann EA, Marques W, et al. Swallowing dysfunction in hereditary neuropathy with liability to pressure palsies. *Arq Neuropsiquiatr*. (2008) 66:898–900. doi: 10.1590/S0004-282X2008000600027

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CASE REPORT
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Case Report: A Patient Diagnosed With Miller Fisher Syndrome and Myasthenia Gravis at the Same Time

*Nan Chen, Hanyu Cai and Jianhua Cheng**

Department of Neurology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

In this case report, we describe a patient who was first diagnosed with Miller Fisher syndrome (MFS) combined with myasthenia gravis (MG). A 58-year-old male patient presented with acute dysarthria with dizziness, ophthalmoplegia, absence of deep tendon reflexes in the extremities, and ataxia. Lumbar puncture 1 week after onset showed albuminocytologic dissociation and serum antibodies against GQ1b and GT1a turned out to be positive. Ultimately, the patient was diagnosed with MFS, which is a rare variant of Guillain-Barre syndrome. Because the clinical manifestations of the patient could not exclude MG, electromyography, and serum muscle weakness antibody profile were performed. The results showed positive for axillary nerve repetitive electrical stimulation and antibodies against acetylcholine receptor (AChR) and titin were detected, so the patient was diagnosed with MG at the same time. Even though only five cases of overlapping MFS and MG so far have been described, two different autoimmune diseases may coexist. When one disease presents with uncommon symptoms, careful identification of the presence or absence of other comorbid diseases should be required.

Keywords: Miller Fisher syndrome, myasthenia gravis, GQ1b, GT1a, titin

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University of Milan, Italy

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Nils Erik Gilhus,
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Elif Kocasoy Orhan,
Istanbul University, Turkey

****Correspondence:***
Jianhua Cheng
chengjianhua@wmu.edu.cn

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BACKGROUND

Miller Fisher syndrome (MFS) is a rare variant of Guillain-Barre syndrome (GBS), an acute, immune-mediated, monophasic disease that usually presents as ocular muscle paralysis, dysreflexia, and ataxia. The worldwide incidence of GBS is estimated to be 1–2 per 100,000 people. Miller-Fischer syndrome accounting for only a small fraction of the total and its prevalence is higher in Asia where it is estimated to account for 15–25% of GBS, compared to only 1–7% in the West (1). Autoimmune myasthenia gravis (MG) is an antibody-mediated chronic disease which is one of the most common disorders affecting neuromuscular transmission, in which alterations in neuromuscular transmission lead to skeletal muscle weakness and fatigability. The worldwide incidence is estimated to be between 0.3 per 100,000 people and 2.8 per 100,000 people (2). The incidences of both diseases are low, so the chance of overlap between the two diseases is very low, with only 5 cases reported worldwide (3–7).

CASE REPORT

A 58-year-old male patient had a sudden onset of dizziness with slurred speech, subsequently accompanied by numbness of upper extremities, choking and coughing with water, and ptosis of the

right eye. Then, he went to the local hospital for consultation, and the brain MRI scan did not show any obvious abnormal signs. In order to determine further treatment, the patient was admitted to our hospital diagnosed with "ball palsy to be investigated."

He previously had "kidney stone surgery" 3 years ago and was diagnosed with nephritis which improved after taking medication (unknown) in March 2021. In April 2021, he underwent "back lipoma resection." The patient had not been previously exposed to the novel coronavirus and had not been vaccinated against it.

At the time of admission, the neurological examination showed ptosis of the right eye and limitation in abduction and adduction of both eyes, absent deep tendon reflexes in the extremities, positive at finger-to-nose test together with a slightly shallowness of nasolabial fold on the right side, weakness of cheek puffing on the right side, slight weakness of the neck extensor muscle, and positive of the right eyelid fatigue test. Bilateral frontal lines are basically symmetrical, tongue extension was center, muscle strength and muscle tone in the limbs was normal, deep and superficial sensation were normal, and there were negative pathological signs on both sides. Brain MRA, chest CT, carotid ultrasound, and vertebral artery ultrasound did not show any lesions related to symptoms. Blood tests were unremarkable except for mild hypercholesterolemia and high red blood cells in the urine. Tumor indicators, autoimmune antibodies, thyroid function, serum folate, and vitamin B12 dose were normal.

The symptoms of the patient were more consistent with the MFS triad. Electromyography, performed 6 days after the onset of the disease, showed axonal damage to peripheral sensory nerves of the extremities. The lumbar puncture performed 1 week after his onset of the disease showed that the cerebrospinal fluid was suggestive of cellular protein separation, and was positive for antibodies against GQ1b and GT1a in 12 items of the serum anti-ganglioside antibodies. Therefore, the diagnosis of Miller-Fisher syndrome was confirmed. On the seventh day after onset, relevant contraindications were excluded, and the patient was given immunoglobulin therapy at 0.4 mg/kg per day for 5 days. The symptoms of the patient improved significantly after one treatment period.

The patient showed mild facial palsy, dysphagia, and weakness of curved neck, which are not common symptoms of MFS. Combined with the fact that the muscle weakness of the

TABLE 1 | Characters of the five cases of overlapping MFS and MG.

	Age(y)	Gender	History of antecedent infection	Clinical presentation	Past history	MG antibodies	MFS antibodies	Treatment	Prognosis
Case 1 (3)	40	Male	2 weeks Had a flu-like illness	Complete ocular muscle paralysis in both eyes, partial ptosis in the left eye, and loss of pupillary light reflex in the right eye. With loss of tendon reflexes, ataxia	MG 7 years	-	anti-GQ1b antibodies	3 times plasma exchange	Good
Case 2 (4)	69	Female	None	Bilateral ptosis with dysarthria after 1 week. Ataxia, loss of tendon reflexes.	Diagnosed with chronic kidney disease 2 years ago	Anti-AchR antibodies	Anti-GQ1b antibodies	First treated with immunoglobulin injections, no improvement in symptoms, then steroid hormone therapy with better results	Better
Case 3 (5)	84	Female	With upper respiratory tract infection 5 days ago	Ptosis, diplopia, dysphagia, and slurred speech, loss of all tendon reflexes	MG 6 year	Anti-AchR antibodies	Anti-GQ1b antibodies	IVIG	Better
Case 4 (6)	79	Male	Upper respiratory tract infection, influenza vaccination received a few weeks ago	Diplopia aggravated with nausea, vomiting, ataxia, loss of tendon reflexes	MG 8 years, mild medical chronic sensorimotor axonal polyneuropathy due to postoperative chemotherapy for colon cancer	Anti-RyR antibodies, anti-AchR antibodies	anti-GQ1b antibodi-es	5plasmapheresis	Better
Case 5 (7)	43	Male	None	Bilateral diplopia, bilateral hand sensory abnormalities, mostly absent tendon reflexes	MG diagnosed 15 years ago as anti-AchR antibody negative	None	Anti-GQ1b antibody	–	Good

patient was fatigue-related and the phenomenon of "light in the morning and heavy in the evening" and positive of the right eyelid fatigue test, MG was excluded. After admission, the chest CT revealed that no significant abnormality could be seen in the thymus gland. Neostigmine test was performed prior to Immunoglobulin treatment, but the results suggested that there was no significant improvement before and after the injection. Despite this, electromyography performed 13 days after the onset of the disease showed that when the axillary nerve was repeatedly electrically stimulated, the wave amplitude decreased by about 35.8% under low frequency electrical stimulation (3 Hz) and about 50.6% under high frequency electrical stimulation (10 Hz). The patient underwent repetitive electrical stimulation tests of the facial and axillary nerves twice within 1 week of admission, both of which were negative for the facial nerve and positive for the axillary nerve. Together with positive of antibodies against acetylcholine receptor (AChR) and titin, the diagnosis of myasthenia gravis was confirmed.

After a course of immunoglobulin injection, the ophthalmoplegia and dysarthria of the patient significantly improved compared with before. At the time of discharge, the patient still had a little numbness in double hands, no obvious slurred speech, no dizziness, and no headache. Neurological examination signs were significantly less severe than before.

DISCUSSION

Together with the five previously published cases of co-morbid MFS and MG, this is the second case in which both diseases were diagnosed simultaneously for the first time, with the first case being published in 2016 (4). The details of the five previous cases are shown in **Table 1**.

In this case, the patient was positive for serum antibodies against GQ1b and GT1a in the 12 anti-ganglioside antibodies. Anti-GQ1b antibodies can cross-react with anti-GT1a antibodies in MFS. GT1a is specifically distributed in the lower part of the central nervous system and is associated with medullary paralysis. Therefore, cross-reactivity between anti-GQ1b and GT1a can lead to medullary paralysis (8). In addition, our patient presented with symptoms such as weakness in swallowing and choking on drinking water. The serum MG profile of our patient showed positive of antibodies against AChR and titin. Notably, anti-Titin antibody IgG is an antibody against intracellular components of rhabdomyocytes (9). It is not clear whether these four antibodies will appear to cross-react with each other, but there is a view that anti-ganglioside antibodies may cause lesions of the neuromuscular junction, leading to the development of muscle weakness symptoms similar to those of MG (10).

Although our patient had nephritis and surgery, antibiotics and anesthesia may have caused MG, but it did not manifest as muscle weakness at that time. Antibiotics and anesthetic supplies can all cause worsening of MG. The mechanisms of antibiotic-induced MG exacerbation include presynaptic interactions with voltage-gated calcium channels, calcium-sensitive receptors, and postsynaptic interactions with acetylcholine receptors (11). Many anesthetic supplies can also directly aggravate MG. Because the variety and dosage of anesthetics during surgery have a great impact on MG patients, anesthetic drugs that do not affect neuromuscular conduction and respiratory function should be selected for MG patients whenever possible (12).

Because both MG and MFS are rare autoimmune mediated diseases, The simultaneous diagnosis of these two diseases in one patient is a rare finding. We should distinguish carefully in the differential diagnosis of neurological diseases in the future and should not ignore the overlap of diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

NC contributed to the conception and drafting of the manuscript. HC contributed to acquisition of data. JC contributed to the drafting and revision of the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Gupta SK, Jha KK, Chalati MD, Alashi LT. Miller Fisher syndrome. *BMJ Case Rep.* (2016) 2016:bcr2016217085 doi: 10.1136/bcr-2016-217085
2. Mantegazza R, Bernasconi P, Cavalcante P. Myasthenia gravis: from autoantibodies to therapy. *Curr Opin Neurol.* (2018) 31:517–25. doi: 10.1097/WCO.0000000000000596
3. Mak W, Chan KH, Ho SL. A case of ocular myasthenia gravis and Miller–Fisher syndrome. *Hosp Med.* (2005)66:116–7. doi: 10.12968/hmed.2005.66.2.17562
4. Tanaka Y, Satomi K. Overlap of myasthenia gravis and miller fisher syndrome. *Intern Med.* (2016) 55:1917–8. doi: 10.2169/internalmedicine.55.6262
5. Lau KK, Goh KJ. The co-occurrence of serologically proven myasthenia gravis and Miller Fisher/Guillain Barré overlap syndrome—A case report. *J Neurol Sci.* (2009) 276:187–8. doi: 10.1016/j.jns.2008.08.019
6. Brusa RB, Faravelli I. Ophthalmoplegia due to miller fisher syndrome in a patient with myasthenia gravis. *Front Neurol.* (2019) 10:823. doi: 10.3389/fneur.2019.00823
7. Silverstein MP,Zimnowodzki S,Rucker JC. Neuromuscular junction in Miller Fisher syndrome. *Semin Ophthalmol.* (2008) 23:211–3. doi: 10.1080/08820530802049996
8. Wanleenuwat P, Iwanowski P, Kozubski W. Antiganglioside antibodies in neurological diseases. *J Neurol Sci.* (2020) 408:116576. doi: 10.1016/j.jns.2019.116576
9. Stergiou C, Lazaridis K, Zouvelou V. Titin antibodies in "seronegative" myasthenia gravis – a new role for an old antigen. *J Neuroimmunol.* (2016) 292:108–15. doi: 10.1016/j.jneuroim.2016.01.018
10. Santafé MM, Sabaté MM, Garcia N. Changes in the neuromuscular synapse induced by an antibody against gangliosides. *Ann Neurol.* (2005) 57:396–407. doi: 10.1002/ana.20403
11. Bhattacharyya S, Darby R, Berkowitz AL. Antibiotic-induced neurotoxicity. *Curr Infect Dis Rep.* (2014) 16:448. doi: 10.1007/s11908-014-0448-3
12. Blichfeldt-Lauridsen L, Hansen BD. Anesthesia and myasthenia gravis. *Acta Anaesthesiol Scand.* (2012) 56:17–22. doi: 10.1111/j.1399-6576.2011.02558.x

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CASE REPORT
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Case Report: Thymidine Kinase 2 (TK2) Deficiency: A Novel Mutation Associated With Childhood-Onset Mitochondrial Myopathy and Atypical Progression

Arianna Manini[1], *Megi Meneri*[1,2], *Carmelo Rodolico*[3], *Stefania Corti*[1,2], *Antonio Toscano*[3], *Giacomo Pietro Comi*[1,4], *Olimpia Musumeci*[3]* *and Dario Ronchi*[1]*

[1] *Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy,* [2] *Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy,* [3] *Unit of Neurology and Neuromuscular Disorders, Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy,* [4] *Neuromuscular and Rare Diseases Unit, Department of Neuroscience, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*

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Edited by:
Edoardo Malfatti, Hôpitaux Universitaires Henri Mondor, France

Reviewed by:
Catarina M. Quinzii, Columbia University, United States
Michelangelo Mancuso, University of Pisa, Italy

***Correspondence:**
Dario Ronchi
dario.ronchi@unimi.it
Olimpia Musumeci
olimpia.musumeci@unime.it

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The nuclear gene *TK2* encodes the mitochondrial thymidine kinase, an enzyme involved in the phosphorylation of deoxycytidine and deoxythymidine nucleosides. Biallelic *TK2* mutations are associated with a spectrum of clinical presentations mainly affecting skeletal muscle and featuring muscle mitochondrial DNA (mtDNA) instability. Current classification includes infantile- (≤1 year), childhood- (1–12 years), and late-onset (≥12 years) forms. In addition to age at onset, these forms differ for progression, life expectancy, and signs of mtDNA instability (mtDNA depletion vs. accumulation of multiple mtDNA deletions). Childhood-onset TK2 deficiency typically causes a rapidly progressive proximal myopathy, which leads to wheelchair-bound status within 10 years of disease onset, and severe respiratory impairment. Muscle biopsy usually reveals a combination of mitochondrial myopathy and dystrophic features with reduced mtDNA content. Here we report the case of an Italian patient presenting childhood-onset, slowly progressive mitochondrial myopathy, ptosis, hypoacusis, dysphonia, and dysphagia, harboring the *TK2* variants c.278A>G and c.543del, the latter unreported so far. Compared to other childhood-onset *TK2*-patients, our case displays atypical features, including slowly progressive muscle weakness and absence of respiratory failure, which are usually observed in late-onset forms. This report extends the genetic background of TK2-related myopathy, highlighting the clinical overlap among different forms.

Keywords: thymidine kinase 2, TK2, mitochondrial DNA, mtDNA maintenance defects, myopathy, deoxynucleosides

INTRODUCTION

Mitochondrial DNA (mtDNA) maintenance defects are a heterogeneous group of clinical syndromes characterized by mtDNA deletions and/or depletion and derived from mutations in nuclear genes variably involved in mtDNA homeostasis (i.e., *POLG1*, *POLG2*, *TWNK*, *DGUOK*, *TYMP*) (1–5).

In 2001, Saada et al. (6) detected biallelic mutations in the thymidine kinase 2 (*TK2*) gene in four children presenting severe myopathy associated with muscle mtDNA depletion. TK2 is a nuclear-encoded mitochondrial enzyme involved in the phosphorylation of deoxycytidine and deoxythymidine nucleosides, which represents the first step of a salvage pathway aimed to provide deoxyribonucleotides (dNTPS) for mtDNA synthesis (7). Recessive *TK2* mutations are now an established cause of mtDNA maintenance disorders, recently classified on the basis of clinical and biochemical features (8). According to age at onset, TK2 deficiency can lead either to an infantile-onset ($\leq$1 year), childhood-onset (1–12 years) and late-onset ($\geq$12 years) myopathy, which differ for rate of weakness progression, post-onset survival and predominance of mtDNA multiple deletions or depletion in muscle tissue, as summarized in **Table 1** (8). Childhood-onset TK2 deficiency is typically associated with rapidly progressive, proximal myopathy, which leads to wheelchair-bound status within 10 years of disease onset in most patients, and post-onset survival longer than 13 years (8). More than half of childhood-onset TK2 patients require ventilatory support due to respiratory impairment (8). Ptosis, chronic external ophthalmoplegia (CPEO), facial weakness and dysphagia have been reported less frequently in this group of patients compared to late-onset cases (8–11), while cognitive decline, encephalopathy, seizures, and non-muscle manifestations are rare compared to the infantile-onset form (8–10). Needle electromyography (EMG) examination frequently evidences myopathic changes [i.e., polyphasic, short-duration, low-amplitude motor unit potentials (MUPs)] (8). Muscle histology reveals a combination of mitochondrial dysfunction [i.e., cytochrome C oxidase (COX)-negative fibers; ragged-red fibers (RRF)] and, mainly in pediatric cases, dystrophic features (i.e., atrophic fibers, fibrosis and increase of connective tissue) (8, 12, 13). Infantile and childhood-onset cases often showed mtDNA depletion in muscle tissue, whereas the late-onset ones are usually associated with mtDNA multiple deletions (8).

Herein, we report the case of an Italian patient affected by a childhood-onset, slowly progressive mitochondrial myopathy with atypical clinical features, harboring two heterozygous *TK2* variants, one of which has not been reported before.

CASE DESCRIPTION

The proband is a 55-year-old Italian woman from Sicily, born to non-consanguineous parents. Mild bilateral ptosis and dysphonia were noticed starting from 8 years of age. At 20 years of age, she started to complain of dysphagia of both solid food and liquids and began losing weight. Over the years she developed bilateral upper limb weakness and mild hypoacusis, with slow progression in the last 20 years. Cognitive abilities are normal.

Current clinical examination reveals bilateral ptosis and ophthalmoparesis, myopathic face, marked dysphonia, wasting, weakness of proximal upper limbs and of psoas muscles, more prominent on the right.

The complete timeline of relevant clinical signs and symptoms and of diagnostic assessments performed during disease progression is reported in **Figure 1**.

Creatine phosphokinase (CK) levels are currently 251 IU/L (normal value $<$ 200). During first hospitalization at 32 years of age, CK levels reached 3,880 IU/L, with concomitant increase of liver enzymes levels, including aspartate aminotransferase (AST) (243 IU/L; normal value $<$43 IU/L), alanine aminotransferase (ALT) (98 IU/L; normal value $<$45 IU/L) and lactate dehydrogenase (LDH) (1,459 IU/L; normal value $<$ 300 IU/L). Lactate levels were only mildly increased at baseline (2.6 mmol/L; normal value $<$ 1.5 mmol/L), but significantly raised under workload (4.6 mmol/L; normal value $<$ 2.3 mmol/L).

Needle EMG examination, performed at 33 years, showed rapid recruitment of short-duration, low-amplitude MUPs in bilateral biceps and first interosseous muscles. Audiometry, electrocardiogram, echocardiogram and spirometry were normal. At the same age, she underwent muscle biopsy of left biceps, which revealed increased fibers size, with coexistence of both atrophic and hypertrophic fibers in addition to mitochondrial dysfunction features, including 10% RRF, succinate dehydrogenase (SDH) hyperactive fibers and COX-negative fibers. The adenosine triphosphatase (ATPase) staining showed marked prevalence of type 1 fibers (almost 90%), and, to a lesser extent, type 2c fibers.

Southern blot analysis and long-range polymerase chain reaction (PCR) of muscle mtDNA showed multiple mtDNA deletions (**Figures 2A,B**). Quantitative PCR showed loss of mtDNA integrity (30% compared to age-matched controls; **Figure 2C**).

After excluding mutations in the common genes associated with multiple mtDNA deletions (*POLG1*, *SLC25A4*, *POLG2*, *TWNK*, *RRMB2B*, *DGUOK*), direct sequencing of *TK2* (NM_004614.4) revealed the heterozygous mutations c.278A>G and c.543del resulting in protein changes p.Asn93Ser and p.Leu182Phefs*11, respectively. The c.278A>G variant [rs142291440; Genome Aggregation Database (gnomAD) Minor Allele Frequency (MAF) 1.2×10^{-6}] has been previously detected in an African American patient affected by childhood-onset mitochondrial myopathy (14). The microdeletion c.543del is absent in publicly available databases. DNA from relatives was not available for molecular studies.

DISCUSSION

We herein describe the case of an Italian patient from Sicily affected with a mild form of childhood-onset mitochondrial myopathy, with muscle mtDNA multiple deletions and depletion, associated with two heterozygous mutations in *TK2*, one of which is novel. To date, this is the second Italian case of *TK2*-related myopathy described so far (15). Intriguingly, both families are of Sicilian origin.

Both the p.Asn93Ser and p.Leu182Phefs*11 variants are located within the deoxynucleoside kinase domain, which

TABLE 1 | Clinical and biochemical features associated with the three different phenotypes of TK2-related mitochondrial myopathy and mtDNA maintenance defects [data were obtained from Garone et al. (8)].

	Infantile-onset	Childhood-onset	Late-onset
Age at onset	≤1 year	1-12 years	≥12 years
Prevalence among TK2-related mitochondrial myopathy cases	43%	41%	16%
Myopathy	Severe, congenital, rapidly progressive	Moderately to rapidly progressive	Subtle signs of myopathy in childhood; slowly progressive; like facioscapulohumeral dystrophy
Ptosis and PEO	8%	30%	69%
Progression to wheelchair-bound status	4 years or no ability to walk (94%)	10 years (63%)	No
Respiratory impairment	+++	++	+
Ventilatory support	89%	55%	44%
Nervous system involvement	26%	11%	0%
Additional neurological features	Seizures (18%) Encephalopathy (13%) Cognitive dysfunction (8%) Facial diplegia (8%) Dysphagia (8%) Lissencephaly (3%) Microcephaly (3%) Bilateral optic atrophy (3%)	Facial diplegia (30%) Hypoacusis (5%) Dysphagia (3%) Cognitive decline (3%) Encephalopathy (3%)	Facial diplegia (43%) Dysphagia (43%) Dysarthria/dysphonia (21%) Peripheral neuropathy (7%) Hypoacusis (7%)
Non-skeletal muscle involvement	33%	19%	25%
Additional non-neurological features (rare)	Multiple bone fractures (5%) Nephropathy (3%) Rigid spine (3%) Cardiomyopathy (3%) Bi-ventricular hypertrophy (3%) Arrhythmia (3%) Esophageal atresia (3%) Anemia (3%) Capillary-leak syndrome (3%) Bilateral chylothorax (3%) Occipital skin necrosis (3%)	Prolonged QT (3%) Arrhythmia (3%) Multiple bone fractures (3%) Renal tubulopathy (3%) Gynecomastia (3%)	Cardiomyopathy (14%)
mtDNA depletion	81%	77%	7%
mtDNA multiple deletions	12.5%	50%	100%
Post-onset survival	1 year	23 years (compound with late-onset cases)	23 years (compound with childhood-onset cases)

MtDNA, mitochondrial DNA; PEO, progressive externa ophthalmoplegia.

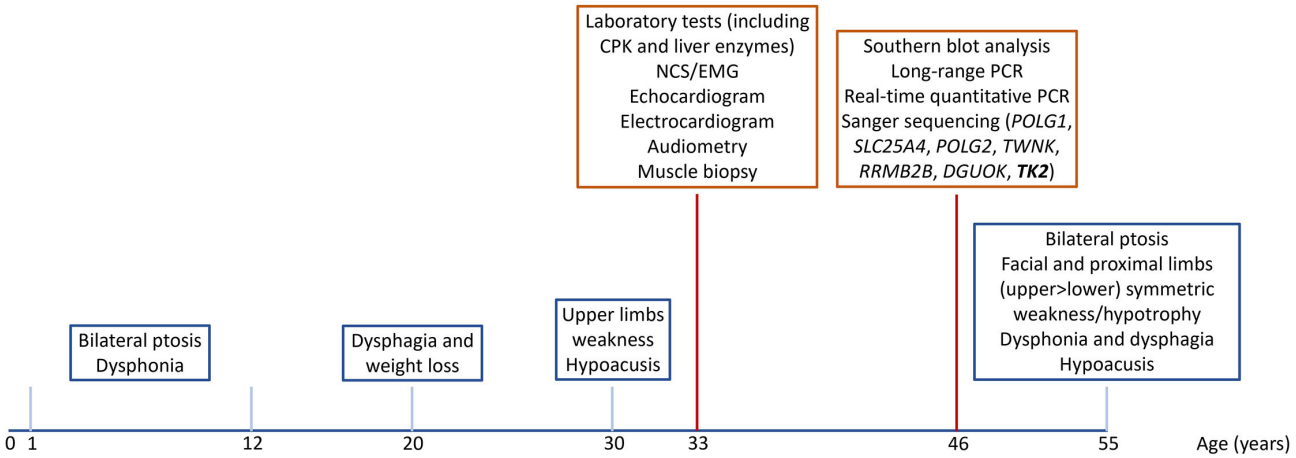

FIGURE 1 | Timeline of relevant clinical signs and symptoms (blue squares), and of diagnostic assessments (red squares).

spans amino acids 53–260. This large functional domain is involved in the phosphorylation of deoxypyrimidine nucleosides, the first step of the mtDNA synthesis cascade (7). The p.Asn93Ser has been already reported by Oskoui et al. (14). Both our proband and the patient described by Oskoui and colleagues display childhood-onset myopathy with progressive development of fatigue, proximal muscle weakness, and facial diplegia, and similar levels of mtDNA depletion assessed in muscle tissue. Main differences between the two cases are represented by the absence of ptosis and the presence of neuropathic changes at EMG reported by Oskoui and colleagues (14). The novel p.Leu182Phefs*11 variant is located in exon 8, which encodes the α8 helix involved in the catalysis of adenosine triphosphate (ATP) molecules due to its capacity of binding phosphate groups. Exon 8 is the second hot spot of *TK2* pathogenic variants, following exon 5 (8). Small indels causing frameshift represent the second most prevalent type of *TK2* variants in mtDNA maintenance defects (13%), following missense mutations (66%), and are distributed throughout *TK2* length (16).

Consistently with classical cases of childhood-onset *TK2* deficiency, analysis of mtDNA levels in muscle tissue of our patient demonstrated both depletion and multiple deletions, and CK levels were mildly elevated (8). Muscle histology findings included non-specific myopathic changes (i.e., fiber size variability, with type 1 predominance), and mitochondrial myopathy markers without dystrophic features. However, several clinical features are atypical compared to previous reports of childhood-onset *TK2*-related myopathy (**Table 1**). First, muscle weakness has remained stable over the last 20 years, and our patient is currently able to walk without support. Childhood-onset *TK2*-related myopathy is usually rapidly progressive, and patients become wheelchair-bound within 10 years of disease onset. In the retrospective analysis of natural history data of genetically confirmed TK2 deficient patients performed by Garone and colleagues, only 4 out of 30 childhood-onset patients were able to walk independently after 10 years from disease onset (8). Of the remaining childhood-onset cases, 19 (63%) became wheelchair-bound within 10 years of disease onset, and 8 (27%) were within 10 years of disease onset, so that their muscle impairment rate of progression could not be assessed. Second, respiratory failure with invasive or non-invasive ventilatory dependency is frequent, reaching about half childhood-onset cases (8). At 55 years of age, our patient has not developed respiratory impairment. Third, dysphagia, which has been described in our case, is rare in childhood-onset *TK2*-related myopathy, especially compared to late-onset forms (8).

As deoxynucleosides therapies for TK2 deficiency are under investigation, identifying patients carrying *TK2* mutations affected by mtDNA maintenance defects becomes pivotal (17). Domínguez-Gonzàlez and colleagues have recently reported the results of an open-label study in which pyrimidine deoxynucleosides and deoxynucleotides were orally administered to 16 patients with mitochondrial myopathy due to TK2 deficiency (17). In early-onset patients affected with severe forms of myopathy, the treatment was effective in increasing survival and ameliorating muscle weakness, respiratory function, and dysphagia, with no major side effects (17). On the contrary, the beneficial effects of the administration of pyrimidine deoxynucleosides and deoxynucleotides in adult-onset cases were limited, and hepatic toxicity was suspected in two patients (17).

The application of NGS-based sequencing in a clinical setting is rapidly expanding the number of novel diagnoses in mitochondrial disorders. On the other hand, the identification of specific changes (such as the coexistence of mtDNA depletion and deletions) might restrict the number of genes to be investigated to those involved in mitochondrial dNTPs supply pathways. Timely or early molecular diagnosis for TK2 patients is crucial for the recruitment in the ongoing

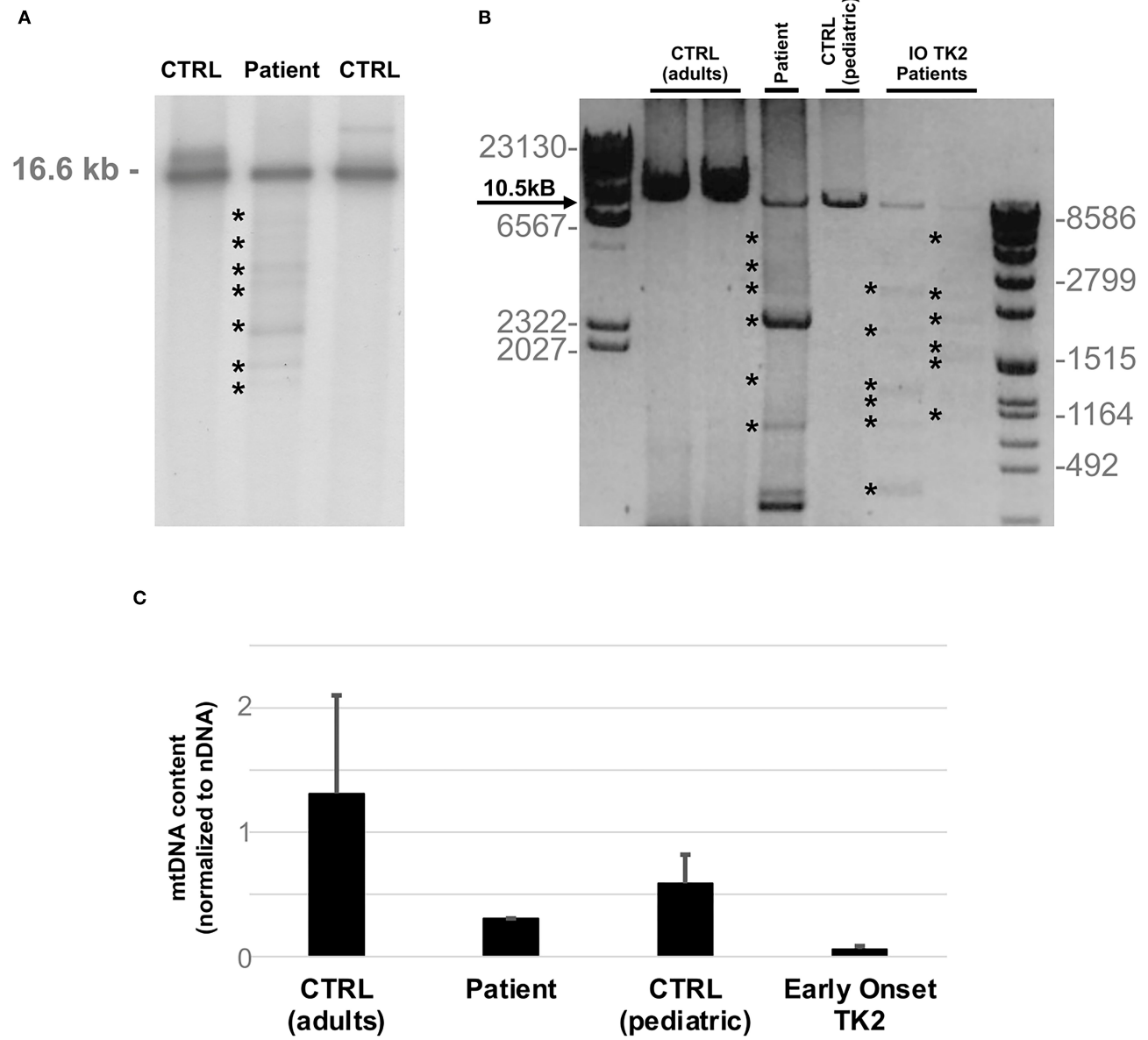

FIGURE 2 | MtDNA studies. **(A)** Southern blot analysis of mtDNA obtained from patient's muscle biopsy and age matched controls. Asterisks indicate multiple bands corresponding to partially deleted mitochondrial genomes. The expected size of normal linearized mtDNA is indicated. **(B)** Long-range PCR analysis of mtDNA obtained from patient's muscle biopsy and two previously described infantile onset TK2 patients (IO TK2) compared to respective age matched controls. Asterisks indicate multiple bands corresponding to multiple mtDNA deletions. Black arrow indicates the expected size of a wild-type PCR amplicon (10.5 kB: FOR5635-RC16135). The sizes of the bands of the ladders (DNA Molecular Weight Marker II and VII, Roche) are indicated. **(C)** Histogram plot showing muscle mtDNA content in patient's muscle and two previously described infantile onset TK2 patients compared to respective age matched controls ($n = 8$, each group). Bars represent mean values. Error bars indicate standard deviation. MtDNA quantification, normalized to nuclear DNA (nDNA) content, was performed by quantitative PCR.

clinical trials and the access to rescue therapies in the near future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Comitato Etico Milano Area 2 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

AM and DR interpreted the results, conceived the idea, revised the literature, and wrote the manuscript. DR performed genetic analysis and mtDNA studies. OM made the clinical evaluation. OM, MM, CR, AT, GC, and SC performed a critical revision of the manuscript for important intellectual content. All the authors have read and approved the manuscript.

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REFERENCES

1. Van Goethem G, Dermaut B, Löfgren A, Martin J-J, Van Broeckhoven C. Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. *Nat Genet.* (2001) 28:211–2. doi: 10.1038/90034
2. Longley MJ, Clark S, Yu Wai Man C, Hudson G, Durham SE, Taylor RW, et al. Mutant POLG2 disrupts DNA polymerase γ subunits and causes progressive external ophthalmoplegia. *Am J Hum Genet.* (2006) 78:1026–34. doi: 10.1086/504303
3. Spelbrink JN, Li F-Y, Tiranti V, Nikali K, Yuan Q-P, Tariq M, et al. Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat Genet.* (2001) 28:223–31. doi: 10.1038/90058
4. Mandel H, Szargel R, Labay V, Elpeleg O, Saada A, Shalata A, et al. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat Genet.* (2001) 29:337–41. doi: 10.1038/ng746
5. Ronchi D, Liu C, Caporali L, Piga D, Li H, Tagliavini F, et al. Novel mutations in DNA2 associated with myopathy and mtDNA instability. *Ann Clin Transl Neurol.* (2019) 6:1893–9. doi: 10.1002/acn3.50888
6. Saada A, Shaag A, Mandel H, Nevo Y, Eriksson S, Elpeleg O. Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy. *Nat Genet.* (2001) 29:342–4. doi: 10.1038/ng751
7. Fasullo M, Endres L. Nucleotide salvage deficiencies, DNA damage and neurodegeneration. *Int J Mol Sci.* (2015) 16:9431–49. doi: 10.3390/ijms16059431
8. Garone C, Taylor RW, Nascimento A, Poulton J, Fratter C, Domínguez-González C, et al. Retrospective natural history of thymidine kinase 2 deficiency. *J Med Genet.* (2018) 55:515–21. doi: 10.1136/jmedgenet-2017-105012
9. Domínguez-González C, Hernández-Laín A, Rivas E, Hernández-Voth A, Sayas Catalán J, Fernández-Torrón R, et al. Late-onset thymidine kinase 2 deficiency: a review of 18 cases. *Orphanet J Rare Dis.* (2019) 14:100. doi: 10.1186/s13023-019-1071-z
10. Tyynismaa H, Sun R, Ahola-Erkkila S, Almusa H, Poyhonen R, Korpela M, et al. Thymidine kinase 2 mutations in autosomal recessive progressive external ophthalmoplegia with multiple mitochondrial DNA deletions. *Hum Mol Genet.* (2012) 21:66–75. doi: 10.1093/hmg/ddr438
11. Alston CL, Schaefer AM, Raman P, Solaroli N, Krishnan KJ, Blakely EL, et al. Late-onset respiratory failure due to TK2 mutations causing multiple mtDNA deletions. *Neurology.* (2013) 81:2051–53. doi: 10.1212/01.wnl.0000436931.94291.e6
12. Collins J, Bove KE, Dimmock D, Morehart P, Wong L-J, Wong B. Progressive myofiber loss with extensive fibro-fatty replacement in a child with mitochondrial DNA depletion syndrome and novel thymidine kinase 2 gene mutations. *Neuromuscul Disord.* (2009) 19:784–7. doi: 10.1016/j.nmd.2009.08.002
13. Vila MR, Segovia-Silvestre T, Gamez J, Marina A, Naini AB, Meseguer A, et al. Reversion of mtDNA depletion in a patient with TK2 deficiency. *Neurology.* (2003) 60:1203–5. doi: 10.1212/01.WNL.0000055928.58122.47
14. Oskoui M, Davidzon G, Pascual J, Erazo R, Gurgel-Giannetti J, Krishna S, et al. Clinical spectrum of mitochondrial DNA depletion due to mutations in the thymidine kinase 2 gene. *Arch Neurol.* (2006) 63:1122. doi: 10.1001/archneur.63.8.1122
15. Galbiati S, Bordoni A, Papadimitriou D, Toscano A, Rodolico C, Katsarou E, et al. New mutations in TK2 gene associated with mitochondrial DNA depletion. *Pediatr Neurol.* (2006) 34:177–85. doi: 10.1016/j.pediatrneurol.2005.07.013
16. Wang J, Kim E, Dai H, Stefans V, Vogel H, Al Jasmi F, et al. Clinical and molecular spectrum of thymidine kinase 2-related mtDNA maintenance defect. *Mol Genet Metab.* (2018) 124:124–30. doi: 10.1016/j.ymgme.2018.04.012
17. Domínguez-González C, Madruga-Garrido M, Mavillard F, Garone C, Aguirre-Rodríguez FJ, Donati MA, et al. Deoxynucleoside therapy for thymidine kinase 2-deficient myopathy. *Ann Neurol.* (2019) 86:293–303. doi: 10.1002/ana.25506

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frontiers in Neurology

CASE REPORT
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Neuropathic Pain as Main Manifestation of *POLG*-Related Disease: A Case Report

Melanie Lang-Orsini[1] *and Paloma Gonzalez-Perez*[2]*

[1] Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States,
[2] Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

Mutations in nuclear-encoded genes that are involved in mitochondrial DNA replication and maintenance (e.g., *POLG*) have been associated with chronic progressive external ophthalmoplegia (CPEO) phenotype. These nuclear genome mutations may lead to multiple mitochondrial DNA deletions or mitochondrial DNA depletion. On the other hand, primary genetic defects of mitochondrial DNA (such as single large-scale deletion or point mutations) have also been associated with the CPEO phenotype. Chronic progressive external ophthalmoplegia (CPEO) may be a manifestation of specific syndromes that, when clinically recognized, prompt clinicians to investigate specific genetic defects. Thus, CPEO, as part of Kearns Sayre syndrome, suggests the presence of a large-scale deletion of mitochondrial DNA. However, in pure CPEO or CPEO plus phenotypes, it is more difficult to know whether causative genetic defects affect the nuclear or mitochondrial DNA. Here, we present a patient with a long-standing history of CPEO plus phenotype, in whom the sequencing of mitochondrial DNA from skeletal muscle was normal, and no other genetic defect was suspected at first. At the time of our evaluation, the presence of polyneuropathy and neuropathic pain prompted us to investigate nuclear genetic defects and, specifically, mutations in the *POLG* gene. Thus, the sequencing of the *POLG* gene revealed p.Thr251Ile and p.Pro587Leu mutations in one allele, and p.Ala467Thr mutation in another allele. Although one would expect that mutations in *POLG* lead to multiple mitochondrial DNA deletions or depletion (loss of copies), the absence of mitochondrial DNA abnormalities in tissue may be explained by heteroplasmy, a lack or no significant involvement of biopsied tissue, or a sampling bias. So, the absence of secondary mitochondrial DNA alterations should not discourage clinicians from further investigating mutations in nuclear-encoded genes. Lastly, mitochondrial point mutations and single mitochondrial DNA deletions very rarely cause CPEO associated with polyneuropathy and neuropathic pain, and POLG-related disease should be considered in this scenario, instead.

Keywords: CPEO, polyneuropathy, neuropathic pain, POLG, mitochondrial disease

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Corrado Italo Angelini,
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***Correspondence:**
Paloma Gonzalez-Perez
pgonzalezperez@partners.org

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INTRODUCTION

The human DNA polymerase gamma is formed by a 140 kDa catalytic subunit called POLG and a 55 kDa dimeric accessory subunit called POLG2. The POLG is encoded by the *POLG* gene (Chr.15q25) and the POLG2 is encoded by the *POLG2* gene (Chr.17q24.1). The POLG has DNA polymerase, 3′ to 5′ exonuclease, and 5′-deoxyribose activities, whereas POLG2 increases the affinity of POLG for mitochondrial DNA. Thus, the DNA polymerase gamma is entirely encoded by nuclear genes, although its main role is the replication of mitochondrial DNA. The *POLG* and *POLG2* mutations may lead to multiple deletions or depletion (loss of copies) of mitochondrial DNA (mtDNA) and have been associated with a broad spectrum of phenotypes such as chronic progressive external ophthalmoplegia (CPEO) (1).

CPEO is a slow, progressive, and painless ocular myopathy characterized by bilateral ptosis and limitation of ocular movements in all directions that is not usually associated with diplopia because, as in other ocular myopathies, no significant misalignment occurs between both eyes. Although other myopathies, such as oculopharyngeal muscular dystrophy, oculopharyngeal distal myopathy, or MHY2-myopathy, are also characterized by progressive ptosis with or without ophthalmoplegia, the term CPEO usually refers to mitochondrial ocular myopathies due to either mutation in mitochondrial or nuclear DNA. The CPEO may occur in isolation (pure CPEO), as part of specific syndromes, or associated with a constellation of clinical manifestations that have not been recognized as syndrome or disorder (CPEO plus). Thus, CPEO is a characteristic feature of Kearns Sayre syndrome (KSS) that is caused by a large-scale 1.1 to 10 kilobase deletion of mtDNA, and it can also be seen as a clinical manifestation of dominantly or recessively inherited *POLG*-related disease; mutations in this nuclear gene account for 25% of patients with CPEO phenotype (2–6).

Here, we report a patient who presented to us for evaluation of polyneuropathy and neuropathic pain as main symptoms, which were previously thought to be unrelated to her long-standing ocular myopathy for which no specific etiology was initially found. Although polyneuropathies are frequent (and commonly idiopathic in the absence of diabetes), they can be a manifestation of a mitochondrial disorder. Furthermore, polyneuropathy has been reported as a predictor of nuclear gene defects in patients with CPEO, and more specifically, the presence of neuropathic pain should prompt the clinician to consider *POLG*-related disease as an unifying diagnosis (7, 8).

CASE DESCRIPTION

A 69-year-old woman was referred to us for paresthesia, neuropathic pain, and cramps in extremities as her main symptoms. She was in good health until her early 50s when she first experienced slowly progressive bilateral ptosis that was surgically corrected twice, and restriction of eye movements in all directions that did not bother her much; she never experienced any diplopia. Eventually, she developed myalgias and fatigue. However, her main complaints were numbness and burning pain in her hands and feet that worsened over time, as well as cramps in her feet. She reported sensitivity to temperature; cold exacerbated burning pain in her feet mostly at night. She also felt clumsy; she frequently dropped things from her hands despite bilateral carpal tunnel release in the past, she had developed mild action hand tremors, and she often suffered near-falls because her balance had deteriorated over time. She was taking duloxetine 30 mg/day and pregabalin 300 mg/day with partial benefit of her burning pain, and she tried lidocaine patches on feet that did not provide any relief. She also reported a long-standing history of dysphagia to solids, with an isolated episode of aspiration in the past and episodes of retrosternal spasms during meals. She denied speech or chewing difficulties. She denied shortness of breath, hearing difficulties, cataracts, or heart problems. Her past medical history also included sleep apnea and cervical and lumbar spine surgeries. She denied alcohol or illicit drug use. Her older brother had similar ocular symptoms and carried a diagnosis of neuropathy; both of uncertain etiology too.

Before the first visit with us, she had undergone several tests since the symptom onset that we have summarized here. Thus, her serum creatine kinase was mildly elevated (268 U/L, ref: 33–211) and her baseline lactate was high (=5.5 nmol/L, ref: 0.5–2.2). Blood cell count, electrolytes, vitamin B12, serum protein electrophoresis, hemoglobin A1C, thyroid-stimulating hormone, C-reactive protein and erythrocyte sedimentation rate, carnitine and acylcarnitine levels, antinuclear antibodies, and acetylcholine receptor binding antibodies were all normal or negative. Urine organic acids were also normal. A brain CT scan did not show any intracranial abnormality. She underwent genetic testing for oculopharyngeal muscular dystrophy that was negative. She had two electrodiagnostic studies; both showed a length-dependent, axonal, and sensory polyneuropathy (sensory nerve action potentials of both sural nerves were absent, and sensory nerve action potentials of ulnar and radial nerves demonstrated reduced amplitudes and normal peak latencies). At the age of 59, she underwent a muscle biopsy of left quadriceps muscle that showed mild myopathic and neuropathic features; the former included occasional subsarcolemmal accumulation of mitochondria and scattered COX-negative muscle fibers, while the latter; angulated fibers, nuclear clumps, and mild fiber type grouping that were attributed to her history of lumbosacral radiculopathy (**Figure 1**). Although such mild mitochondrial findings within the sixth decade of life could be a consequence of normal aging, a manifestation of a mitochondrial disorder was also plausible. Sequencing of mtDNA from muscle tissue did not detect any point mutation or deletions. Furthermore, mitochondrial carnitine and CoQ10 levels, and activity of electron transport chain complexes were all normal from the biopsied muscle tissue. Although no definitive diagnosis was reached at that time, a mitochondrial disorder was still favored and she was started on L-carnitine, creatine, and CoQ10 for that reason.

At initial evaluation with us, approximately 15 years after symptom onset, her exam revealed normal fundoscopic evaluation, normal pupils that were bilaterally reactive to light, symmetric and severe impairment of extraocular motility in all

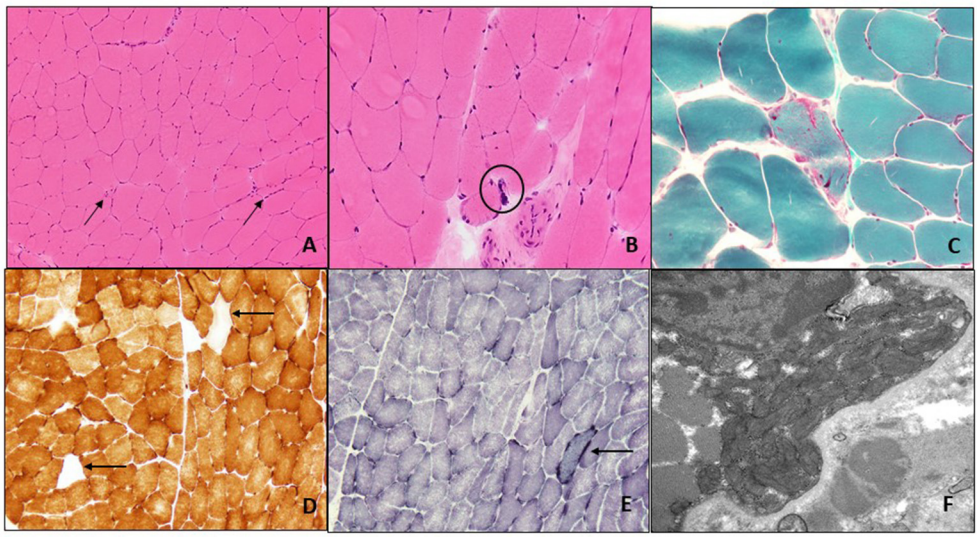

FIGURE 1 | Muscle biopsy of left quadriceps: H&E-stained frozen section of the left quadriceps muscle biopsy (200X) shows occasional angulated atrophic fibers (arrows) and scattered internal nuclei **(A)**. At high power (400X) occasional nuclear bags are seen (circle) **(B)**. Gomori trichrome stain (400X) reveals subsarcolemmal accumulation of mitochondria with an irregular accumulation of Gomori-positive sarcoplasmic material **(C)**. There are scattered COX-negative fibers (arrows) **(D)**, and subsarcolemmal accumulation of mitochondrial is again seen on SDH staining (arrow) **(E)** (both 200X). Electron microscopy (22,000X) shows enlarged and pleomorphic mitochondria that were present in the subsarcolemmal region only **(F)**.

directions of gaze without misalignment, and moderate bilateral ptosis despite past surgical corrections. She did not have facial weakness, her hearing was normal to conversation, and the rest of the cranial nerves were intact. Her muscle strength, bulk, and tone were normal. There were no myotonia or fasciculations. Deep tendon reflexes were 2+ at biceps, brachioradialis, and triceps, and were bilaterally absent at patella and ankles. Plantar responses were flexor. Temperature and pin sensations were severely reduced in feet and hands, proprioception was impaired at great toes, the vibratory sensation was abolished at great toes and malleoli, and Romberg test was positive. There was no dysmetria on finger-to-nose or heel-to-shin tests. She had a mild wide-based gait; she was unable to walk in tandem but able to walk on heels and toes.

An autosomal recessive CPEO plus syndrome, involving the peripheral nerve, was then suspected. The coexistence of polyneuropathy prompted us to investigate nuclear DNA defects that might be causing secondary defects of mtDNA despite no alterations in mtDNA sequencing from the biopsied muscle, which might be explained by heteroplasmy, sampling bias, or lack of significant involvement of skeletal muscle in her case. More specifically, the presence of neuropathic pain pointed to consider a *POLG*-related disease as a possibility; whereas most "mitochondrial neuropathies" are painless, neuropathic pain appears to be more common in patients who have *POLG* mutations (7, 8). Genetic testing of *POLG* gene revealed a known compound of heterozygous mutations: one that comprises two single nucleotides *in-cis* (c.752 C>T and c.1760 C>T, which lead to p.Thr251Ile and p.Pro587Leu, respectively) and another one *in-trans* (c.1399G>A, p.Ala467Thr); these findings confirmed a *POLG*-related disease as unifying diagnosis.

DISCUSSION

Here, we present a patient with painless, progressive, bilateral, adult-onset ptosis and ophthalmoparesis, who also developed symptoms suggestive of peripheral nerve (polyneuropathy and neuropathic pain) and skeletal muscle (myalgias) involvement. This multi-organ phenotype suggested a mitochondrial disorder. An elevated serum lactic acid at baseline increased the diagnostic suspicion for a mitochondrial disorder. Although the presence of occasional subsarcolemmal mitochondrial accumulation and scattered COX-negative fibers on muscle biopsy could be aging-related findings in her case, a manifestation of a mitochondrial disorder could not be ruled out. However, the sequencing of mtDNA from biopsied muscle tissue was normal. In an affected organ (such as skeletal muscle), one would expect a single mtDNA deletion or point mutations of mtDNA in primary mitochondrial disorders, and multiple mtDNA deletions or mtDNA depletion in secondary mitochondrial disorders due to nuclear genetic defects. We suspected that the normal result of mtDNA sequencing from biopsied muscle and the non-specific and mild pathological findings contributed to delay in diagnosis in this case. However, although sequencing of mtDNA may identify deletions and point mutations, it may miss mtDNA depletion (loss of mtDNA copies) that would require assessment of copy number which was not performed. It is also plausible that skeletal muscle was not affected enough to reveal abnormalities of mtDNA (low or no mutant copies of mtDNA to detect), or that muscle sampling missed mtDNA abnormalities, or a combination of both. Thus, normal mtDNA sequencing should not discourage clinicians from further investigating the possibility of a mitochondrial disorder. Furthermore, neuropathy

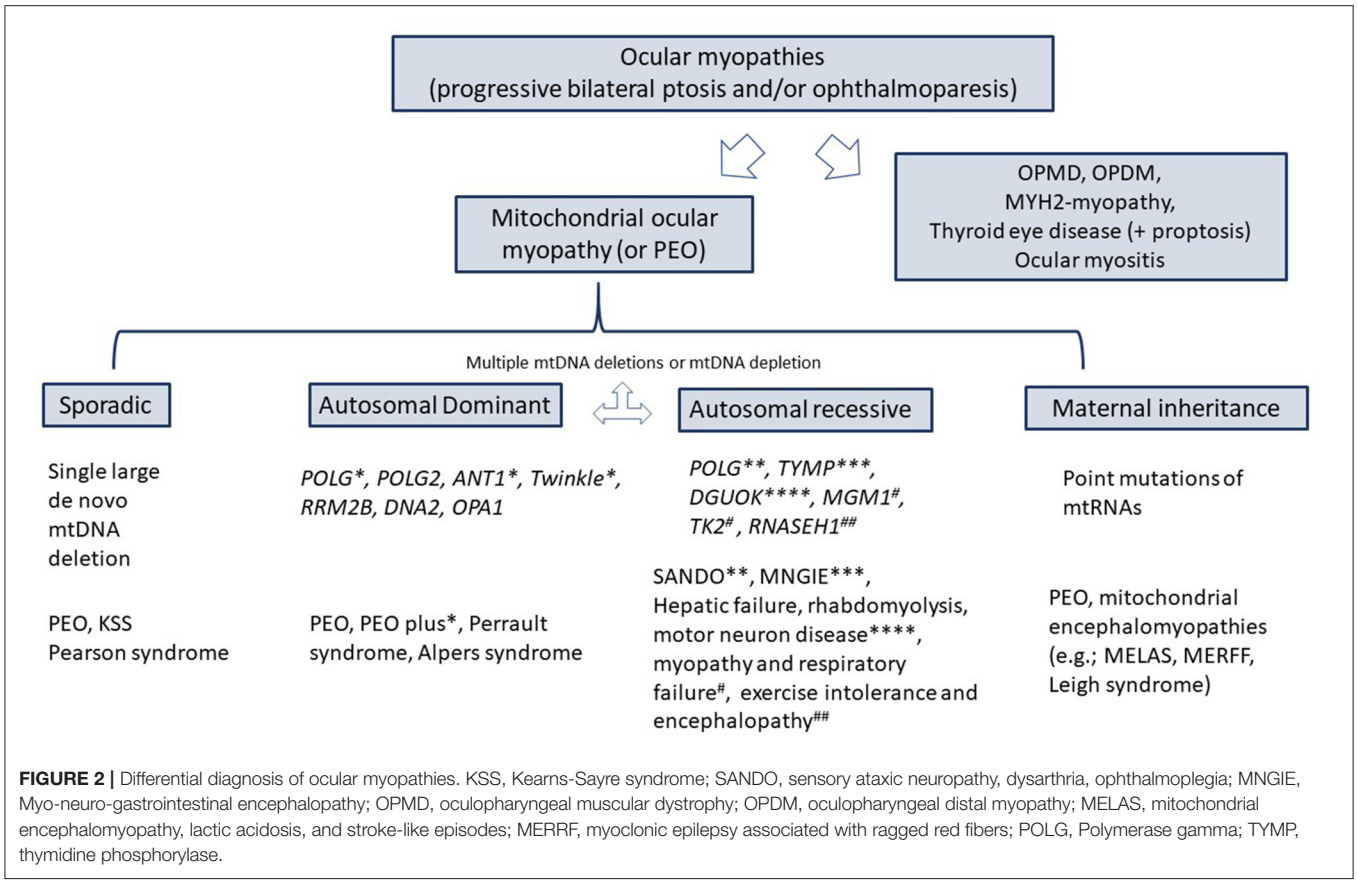

FIGURE 2 | Differential diagnosis of ocular myopathies. KSS, Kearns-Sayre syndrome; SANDO, sensory ataxic neuropathy, dysarthria, ophthalmoplegia; MNGIE, Myo-neuro-gastrointestinal encephalopathy; OPMD, oculopharyngeal muscular dystrophy; OPDM, oculopharyngeal distal myopathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy associated with ragged red fibers; POLG, Polymerase gamma; TYMP, thymidine phosphorylase.

is rare in patients with single large-scale mitochondrial deletions or mtDNA point mutations (4, 12). Similar symptoms in her brother pointed to an autosomal recessive inheritance in her case which increased our suspicion for mutations in a nuclear gene. Lastly, polyneuropathy is a predictor of nuclear genetic defects in mitochondrial disorders, and the presence of neuropathic pain pointed to *POLG* gene as responsible for her phenotype (although most neuropathies associated with mitochondrial disorders are painless, painful neuropathies have been estimated to occur in up to a third of patients with *POLG* mutations) (7–9).

The term CPEO (or PEO) usually applies to mitochondrial ocular myopathy; either due to mutations in mtDNA or nuclear DNA. However, ptosis, with or without ophthalmoparesis of similar characteristics as CPEO, can be seen in non-mitochondrial myopathies, such as oculopharyngeal muscular dystrophy, oculopharyngeal distal myopathy, or MHY2 myopathy (**Figure 2**). It is well known that the phenotypic spectrum of POLG-related disease is broad, and that awareness of such is key to diagnosing this disorder. In 2001, Van Goethem et al. reported the first *POLG* mutations as the cause of the CPEO phenotype (10). The number of *POLG* mutations and their associated clinical manifestations have dramatically expanded over the years (1). Identified *POLG* mutations in this patient (the complex p.Thr251Ile and p.Pro587Leu on one allele and p.Ala467Thr on the second allele) were previously reported in patients with CPEO or CPEO plus syndromes, and with infantile hepatocerebral syndromes (Alpers syndrome) (5, 11).

TABLE 1 | Electrodiagnostic features of "mitochondrial neuropathies" associated with CPEO.

	Syndrome/disease	Nerve conduction studies
1. Mutations in nuclear genes		
POLG	POLG-related disease	Axonal/mixed, mainly sensory PN
	SANDO	
TYMP	MNGIE	Demyelinating/mixed sensory-motor PN
2. Mutations in either nuclear or mitochondrial genes	Leigh syndrome	Demyelinating sensory-motor PN
3. Point mutations of mtDNA	MELAS/MERRF	
		PN not a main feature of phenotype
4. Single large-scale deletion of mtDNA	Kearns-Sayre syndrome	

PN, polyneuropathy.

This patient underwent electrodiagnostic studies twice and a muscle biopsy. An axonal or mixed sensory polyneuropathy has been reported in patients with *POLG* mutations, but this

electrical finding is not specific (12). **Table 1** summarizes the electrical pattern of polyneuropathies in mitochondrial disorders in which CPEO is a hallmark. Likewise, and in addition to mild mitochondrial pathology in muscle fibers on muscle biopsy, the presence of neurogenic changes (angulated fibers, nuclear bags, and fiber type grouping) is also common in mitochondrial disorders but with no specific, and her history of lumbosacral radiculopathy may have also accounted for them (13).

Although polyneuropathy and neuropathic pain are common, a mitochondrial disorder should be considered in the presence of CPEO. Genetic testing in blood samples looking for nuclear defects should be the first step in this scenario. We would like to emphasize the importance of continuing to define and recognize clinical mitochondrial syndromes to avoid unnecessary testing and delays in diagnosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ML-O: drafting and preparation of pathological studies (muscle biopsy). PG-P: design and drafting of the manuscript until final revision. Both authors contributed to the article and approved the submitted version.

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REFERENCES

1. Rahman S, Copeland WC. POLG-related disorders and their neurological manifestations. *Nat Rev Neurol.* (2019) 15:40–52. doi: 10.1038/s41582-018-0101-0
2. McClelland C, Manousakis G, Lee MS. Progressive external ophthalmoplegia. *Curr Neurol Neurosci Res.* (2016) 16:53. doi: 10.1007/s11910-016-0652-7
3. Mancuso M, Orsucci D, Angelini C, Bertini E, Carelli V, Pietro Comi G, et al. Redefining phenotypes associated with mitochondrial DNA single deletion. *J Neurol.* (2015) 262:1301–9. doi: 10.1007/s00415-015-7710-y
4. Yamashita S, Nishino I, Nonaka I, Goto Y. Genotype and phenotype analyses in 136 patients with single large-scale mitochondrial DNA deletions. *J Hum Genet.* (2008) 53:598–606. doi: 10.1007/s10038-008-0289-8
5. Horvath R, Hudson G, Ferrari G, Fütterer N, Ahola S, Lamantea E, et al. Phenotypic spectrum associated with mutations of the mitochondrial polymerase gamma gene. *Brain.* (2006) 129:1674–84. doi: 10.1093/brain/awl088
6. Maghbooli M, Ghaffarpour M, Ghazizadeh T, Shalbaf NA, MalekMahmoudi G. Clinicogenetical variants of progressive external ophthalmoplegia-An especial review of non-ophthalmic manifestations. *Neurol India.* (2020) 68:760–8. doi: 10.4103/0028-3886.293454
7. Horga A, Pitceathly RD, Blake JC, Woodward C, Zapater P, Fratter C, et al. Peripheral neuropathy predicts nuclear gene defect in patients with mitochondrial ophthalmoplegia. *Brain.* (2014) 137:3200–12. doi: 10.1093/brain/awu279
8. Mancuso M, Orsucci D, Angelini C, Bertini E, Carelli V, Pietro Comi G, et al. "Mitochondrial neuropathies": a survey from the large cohort of the Italian Network. *Neuromuscul Disord.* (2016) 26:272–6. doi: 10.1016/j.nmd.2016.02.008
9. Lehmann D, Kornhuber ME, Clajus C, Alston CL, Wienke A, Deschauer M, et al. Peripheral neuropathy in patients with CPEO associated with single and multiple mtDNA deletions. *Neurol Genet.* (2016) 2:e113. doi: 10.1212/NXG.0000000000000113
10. Van Goethem G, Dermaut B, Lofgren A, Martin JJ, Van Broeckhoven C. Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. *Nat Genet.* (2001) 28:211–2. doi: 10.1038/90034
11. Ferrari G, Lamantea E, Donati A, Filosto M, Briem E, Carrara F, et al. Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA polymerase-gammaA. *Brain.* (2005) 128:723–31. doi: 10.1093/brain/awh410
12. Finsterer J. Inherited mitochondrial neuropathy. *J Neurol Sci.* (2011) 304:9–16. doi: 10.1016/j.jns.2011.02.012
13. Laforet P, Lombes A, Eymard B, Danan C, Chevallay M, Rouche A, et al. Chronic progressive external ophthalmoplegia with ragged-red fibers: clinical, morphological and genetic investigations in 43 patients. *Neuromuscul Disord.* (1995) 5:399–413. doi: 10.1016/0960-8966(94)00080-S

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CASE REPORT
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Case Report: Anti-NF186+ CIDP After Receiving the Inactivated Vaccine for Coronavirus Disease (COVID-19)

Shirui Wen, Kailing Huang, Haoyue Zhu, Peihong Li, Luo Zhou and Li Feng*

Department of Neurology, Xiangya Hospital, Central South University, Changsha, China

Corona Virus Disease 2019 (COVID-19), the novel coronavirus disease, is now a global pandemic. Vaccination can significantly reduce the mortality rate caused by the severe acute respiratory syndrome of coronavirus 2 (SARS-CoV-2). There are currently several effective vaccines that have been introduced. Inactivated COVID-19 vaccine is one of these options and is generally considered safe. Neurofascin (NF) plays an important role in keeping the functionality of the node of Ranvier. We report here a rare case of anti-NF186+ chronic inflammatory demyelinating polyneuropathy (CIDP) in a 23-year-old male patient who was vaccinated with inactivated COVID-19 vaccine prior to the onset. This report adds a new possible rare side effect of a COVID-19 vaccine and provides a case for the clinical effectiveness of rituximab (RTX) in patients with anti-NF186+ CIDP.

Keywords: inactivated COVID-19 vaccine, NF186, chronic inflammatory demyelinating polyneuropathy (CIDP), autoimmune disease, COVID-19

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Giovanni Meola,
University of Milan, Italy

Reviewed by:
Elif Kocasoy Orhan,
Istanbul University, Turkey
Samir Abu-Rumeileh,
University Hospital in Halle, Germany

****Correspondence:***
Luo Zhou
zhouluo33@163.com

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INTRODUCTION

Since the end of 2019, the Corona Virus Disease 2019 (COVID-19) caused by the severe acute respiratory syndrome of coronavirus 2 (SARS-CoV-2) has posed a significant threat to the world. The disease is an acute severe respiratory syndrome with florid pulmonary manifestations and multi-organ involvement including cardiovascular, musculoskeletal, gastrointestinal, and neurological complications. Chronic inflammatory demyelinating polyneuropathy (CIDP) is one of the autoimmune disorders of the peripheral nervous system. Vaccination is the most crucial way to contain the COVID-19 pandemic. Inactivated or live-attenuated viruses, as well as recombinant proteins and vectors technologies, have been employed to develop the COVID-19 vaccine. So far, there is no case report of anti-NF186+ CIDP after exposure to the COVID-19 inactivated vaccine. This may be the first case of anti-NF186+ CIDP after receiving vaccination based on our knowledge.

Case Reports

A 23-year-old male was admitted to the Department of Neurology, Xiangya Hospital, Central South University on June 22, 2021, with acute limb weakness and numbness for 28 days. He had received his second dose of inactivated coronavirus vaccine the day before these symptoms appeared. On May 26, 2021, the patient developed weakness of the left upper limb, and subsequently, the symptoms progressed to numbness and weakness of the extremities. On June 12, he went to the local hospital for the examination of cerebrospinal fluid (CSF), which showed increased total protein (0.99 g/L) and normal cell count (4×10^6/L). He was initially diagnosed with Guillain-Barre syndrome (GBS). After the intravenous injection of a human immunoglobulin [0.4g/(kg d)]

from June 16 to 20, there was no significant improvement in the symptoms but gradual aggravation occurred. Therefore, he came to our hospital for treatment. There was no history of any viral or respiratory illness before the symptoms appeared. His past medical history was received wherein it is stated that his first dose of inactivated coronavirus vaccine was on March 28, 2020, and the second dose was on May 25, 2021. There was nothing special about personal and family history.

Physical examination revealed symmetrical upper limb weakness [Medical Research Council (MRC) grade 3/5 distally and 2/5 proximally] and lower limb weakness (MRC grade 2/5 distally and 1/5 proximally) and areflexia. The long glove-like pain sensation below the elbow joint of both upper limbs and the knee joint of both lower limbs decreased. Bilateral pathological signs were not elicited.

Auxiliary Examination

The patient's comprehensive examinations showed that the platelets were low, urine protein was positive, hematuria, complement C3, and C4 were significantly reduced, and many kinds of autoantibodies were positive, such as thyroid antibodies, rheumatic immune antibodies (see **Supplementary Table 1**). Sensory nerve conduction studies (NCS): upper limbs showed reduced sensory nerve action potential amplitudes; the lower limbs were normal. Motor NCS: upper limbs showed severely reduced compound muscle action potential amplitude responses and conduction velocities; the lower limbs showed reduced compound muscle action potential amplitude responses and normal conduction velocities. The tibial F wave and H reflex latencies were prolonged. Needle electromyogram (EMG) displayed neurogenic damage. The MRI of the brain and spine was normal and there was no evidence of central destination on T2 and fluid attenuated inversion recovery (FLAIR) images. Re-examination of cerebrospinal fluid analysis in our hospital showed elevated protein (0.86 g/L) and the normal level of leucocytes (3×10^6 L). The patient was diagnosed with immune-associated peripheral neuropathy on admission, and GBS was most likely to be considered. The patient began to receive methylprednisolone pulse therapy.

In the course of treatment, the patient's limb weakness gradually aggravated, manifested as symmetrical limb weakness (MRC grade 0/5 distally and 0/5 proximally), and slowly involving the cranial nerves, like inadequate bilateral eyeball abduction, diplopia. On July 2, the patient's condition further deteriorated. He developed dyspnea with type II respiratory failure and was transferred to the intensive care unit. During the treatment, the tracheotomy ventilator was given to assist breathing, and the high dose of intravenous injection of glucocorticoid was gradually reduced to an oral low dose for maintenance. The patient received plasma exchange on July 9 and 14, respectively. However, his condition did not improve significantly.

To further confirm the diagnosis, the B cell subset test *via* Flow cytometric immunofluorescence assay (FIFA) showed 7.83% CD20+ lymphocytes (see **Supplementary Figure 1**), and a serum antibody test (Cell-based assay, **Figure 1**) displayed anti-NF186 antibodies IgG 1:100. Then, we decided to let him stop taking oral prednisone and gave him rituximab with a total of 600 mg to suppress immunity along with other symptomatic and supportive treatments. The patient's condition was better than before. A week later, the B cell subsets examination showed 0.04% CD20+ lymphocytes (see **Supplementary Figure 2**) and the anti-NF186 antibodies IgG was negative (**Figure 1**).

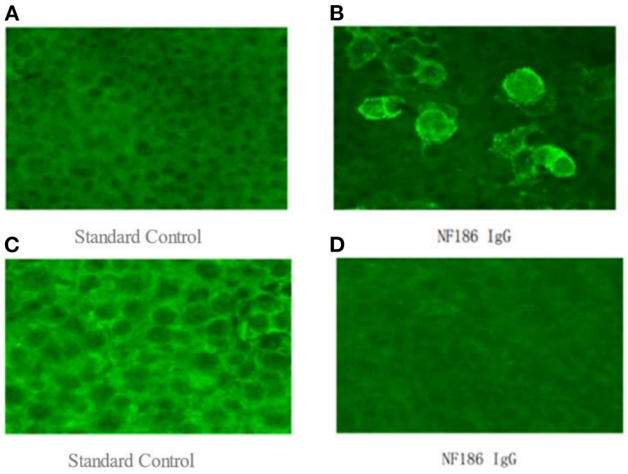

FIGURE 1 | (A,B) a serum antibody test (Cell-based assay), which was examined on July 26, 2021, displayed anti-NF186 antibodies IgG 1:100. **(C,D)** the anti-NF186 antibodies IgG, which was examined on August 12, 2021, was negative after rituximab (RTX) treatment.

Outcome and Follow Up

In August, the patient was diagnosed with immune-associated peripheral neuropathy, most likely to be anti-NF186+ CIDP. The patient has stopped using the ventilator, limb weakness and numbness have improved, no limb pain, and no dyspnea. A physical examination showed normal eye movement, symmetrical upper limb weakness (MRC grade 3/5 distally and 2/5 proximally), lower limb weakness (MRC grade 2/5 distally and 1/5 proximally), and areflexia. Muscle atrophy could be seen in the extremities, and the pathological signs were not elicited. In September, this patient was able to walk independently. Physical examination showed symmetrical upper and lower limb weakness (MRC grade 4/5 distally and 4/5 proximally, **Figure 2**).

DISCUSSION

The COVID-19 infection is a serious, complicated, and widespread disease; other than the respiratory symptoms, it is usually accompanied by a host of neurological complications. The wide spectrum of neurological complications includes cranial neuropathies with anosmia and dysgeusia, stroke, meningitis, and encephalitis. In addition, peripheral neuropathy, such as the GBS and CIDP, was reported in patients with COVID-19 infection (1–3). The GBS usually occurs following the infection, however, it has also been reported to occur after vaccination, surgery, or the administration of immune checkpoint inhibitors.

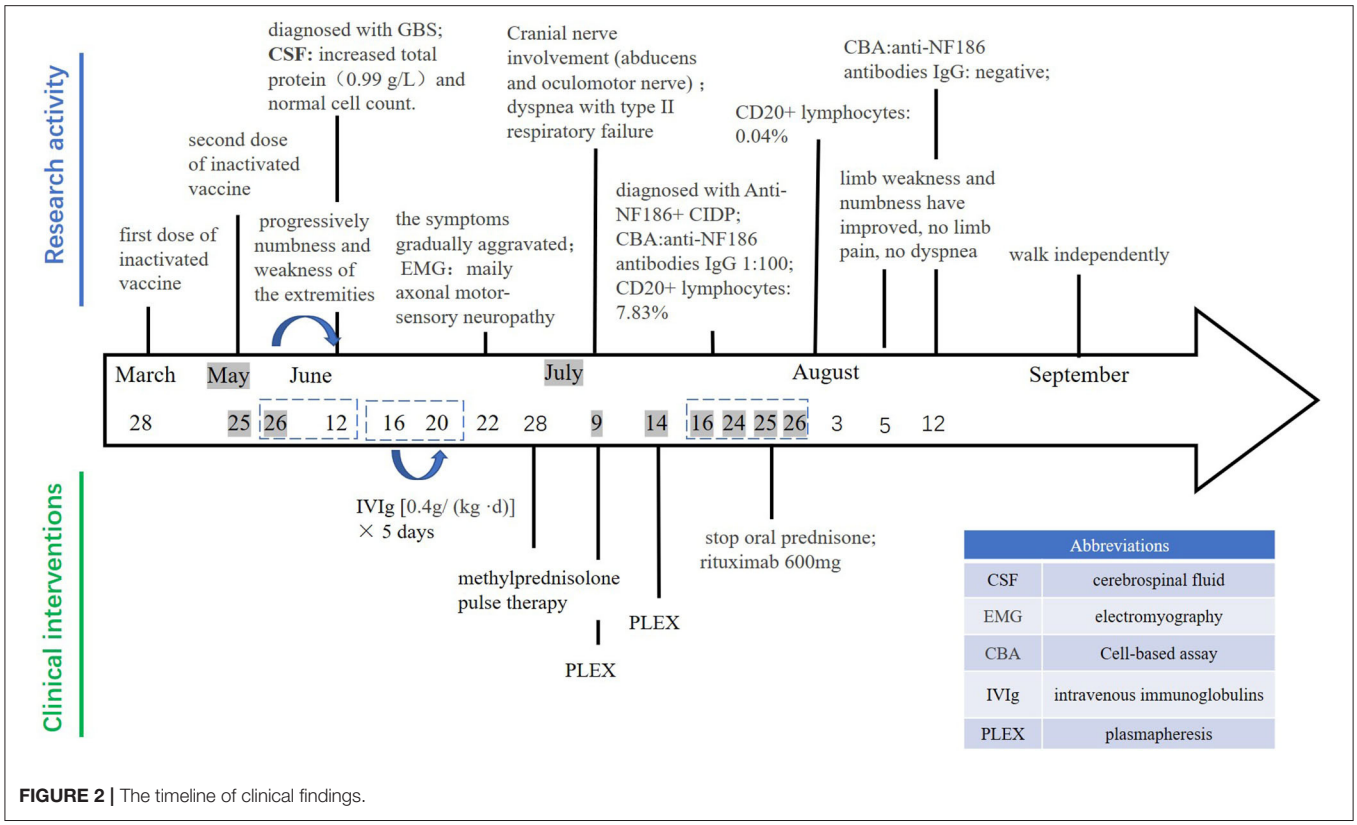

FIGURE 2 | The timeline of clinical findings.

The safety profile of the inactivated vaccine for COVID-19 is good with commonly reported mild side effects such as pain at the injection site, allergic reactions on the skin, flu-like symptoms, headaches, fatigue, and so on (4). Post-vaccination neuropathies are rare events, and CIDP developing during the post-vaccination period is significantly unusual. To date, only two patients who developed CIDP after COVID-19 vaccination were reported, with a typical gradual onset of ascending lower limb weakness and sensory changes; one with facial involvement, another without (5). Therefore, our report extends the possible outcomes in patients who develop CIDP following COVID-19 vaccination and recommends that these patients need close monitoring after the acute phase to rule out the chronic evolution of the disease, which is critical for long-term treatment.

Our patient developed early-onset symptoms mimicking typical GBS with acute limb numbness and weakness after the inactivated COVID-19 vaccine. However, about 8 weeks later, his clinical symptoms continued to deteriorate, and cranial nerve involvement such as ophthalmoplegia appeared. Thus, after positive detection of NF-186 antibody, a diagnosis of NF186+ CIDP was confirmed. The CIDP is a common acquired immune-mediated peripheral neuropathy with strong clinical heterogeneity, including various clinical manifestations, and different responses to the same treatment. In recent years, early research found that autoantibodies against NF, contactin1 or contactin-associated protein 1 (Caspr) could be identified in ∼10% of patients with CIDP. The pathology caused by these antibodies is called nodopathy-paranodopathy, unlike seronegative CIDP, which is no overt inflammation and demyelination and is characterized by dissection of myelin loops from axon at the paranode and subsequent axonal degeneration. In addition, patients with CIDP of this type typically respond poorly to IVIg but may benefit from plasmapheresis and rituximab (RTX) (6). These features are consistent with the clinical presentation of our patients. In our patient, electromyography displayed mainly axonal motor-sensory neuropathy and was accompanied by demyelinating damage. Moreover, he only responded to rituximab therapy.

In the blood test, our patient was found to have positive anti-nRNP/sm antibody, anti-nuclear antibody with 1:320 homogeneous + cytoplasmic granular type, and anti-double-stranded DNA. The complement C3 and C4 decreased. The urine routine showed a positive urine protein. Although the patient has no clinical manifestations, he met the diagnostic criteria for asymptomatic systemic lupus erythematosus (SLE). We speculate that the underlying disease SLE may induce the development of anti-NF186+ CIDP after vaccination.

We are admittedly aware of the lack of a biological marker to establish causality between anti-NF186+ CIDP and the vaccine. However, we cannot ignore the dramatic temporal association between receiving the vaccine and developing severe ophthalmoplegia, the prominent symptoms of dyspnea in a previously healthy male. Moreover, though the specific mechanism remains unknown, the hypothetical triggers for the pathogenesis of autoimmune disorders of the peripheral nervous system such as the Guillain–Barré syndrome (GBS)

(1), acute inflammatory demyelinating polyneuropathy (AIDP), and CIDP (3, 7, 8) are viruses or viral vaccines. Some studies indicated it may be viewed as a result of the interaction between the susceptibility of the vaccinated subject and various vaccine components. Molecular mimicry is one of the implicated mechanisms, which refers to a significant similarity between certain pathogenic elements contained in the vaccine and specific human proteins (5, 9). Previous studies mentioned the potential for cross-reactivity between the coronavirus spike protein target produced by the messenger RNA (mRNA) vaccine and myelin basic protein (MBP) antigens. Moreover, adjuvants contained in vaccines (used mainly to increase the response to vaccination in the general population) may play a role in producing diverse autoimmune and inflammatory responses (10). The possible interactions between genetic predisposition, a history of other autoimmune conditions, the presence of adjuvants, and cross-reactivity between spike proteins and MBP antigens may all contribute to the genesis of peripheral neuropathy and require further investigation (9, 11).

CONCLUSION

We report a rare case of anti-NF186+ CIDP after the second dose of inactivated COVID-19 vaccine. We attribute the occurrence of anti-NF186+ CIDP to the vaccine due to the temporal relationship and the lack of risk factors for CIDP in the patient. This report adds to the literature a possible rare side effect of a COVID-19 vaccine and contributes to the extremely limited literature on potential neurological side effects of inactivated vaccines. Healthcare providers should be aware of the possibility of post-vaccination CIDP. The patient had progressively aggravated limb weakness that was refractory to immunotherapy with pulse steroids and plasmapheresis, and with a dramatic response to RTX. This likely reflects an underlying autoimmune mechanism in the anti-NF186+ CIDP. Further research is needed to probe and study the exact mechanism at a more molecular level.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

KH performed the systematic literature search and made figures. SW drafted the complete manuscript. HZ and PL collected the clinical data. LZ and LF revised the manuscript for important intellectual content. All authors gave the final approval of the final version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.838222/full#supplementary-material

REFERENCES

1. Abu-Rumeileh S, Abdelhak A, Foschi M, Tumani H, Otto M. Guillain-Barré syndrome spectrum associated with COVID-19: an up-to-date systematic review of 73 cases. *J Neurol.* (2021) 268:1133–70. doi: 10.1007/s00415-020-10124-x
2. Caress JB, Castoro RJ, Simmons Z, Scelsa SN, Lewis RA, Ahlawat A, et al. COVID-19-associated Guillain-Barré syndrome: the early pandemic experience. *Muscle Nerve.* (2020) 62:485–91. doi: 10.1002/mus.27024
3. Van Looy E, Veenker L, Steyaert A, Leenders J, Malfroid G, De Cauwer H. COVID-19-induced exacerbation of chronic inflammatory demyelinating polyneuropathy. *J Neurol.* (2021) 268:3129–31. doi: 10.1007/s00415-021-10417-9
4. Xia S, Duan K, Zhang Y, Zhao D, Zhang H, Xie Z, et al. Effect of an inactivated vaccine against SARS-CoV-2 on safety and immunogenicity outcomes: interim analysis of 2 randomized clinical trials. *J Am Med Assoc.* (2020) 324:951–60. doi: 10.1001/jama.2020.15543
5. Bagella CF, Corda DG, Zara P, Elia AE, Ruiu E, Sechi E, et al. Chronic inflammatory demyelinating polyneuropathy after ChAdOx1 nCoV-19 vaccination. *Vaccines.* (2021) 9:121502. doi: 10.3390/vaccines 9121502
6. Vural A, Doppler K, Meinl E. Autoantibodies against the node of ranvier in seropositive chronic inflammatory demyelinating polyneuropathy: diagnostic, pathogenic, and therapeutic relevance. *Front Immunol.* (2018) 9:1029. doi: 10.3389/fimmu.2018.01029
7. Abu-Rumeileh S, Garibashvili T, Ruf W, Fangerau T, Kassubek J, Althaus K, et al. Exacerbation of chronic inflammatory demyelinating polyneuropathy in concomitance with COVID-19. *J Neurol Sci.* (2020) 418:117106–117106. doi: 10.1016/j.jns.2020.117106
8. Beghi E, Feigin V, Caso V, Santalucia P, Logroscino G. COVID-19 infection and neurological complications: present findings and future predictions. *Neuroepidemiology.* (2020) 54:364–9. doi: 10.1159/000508991
9. Vojdani A, Kharrazian D. Potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases. *Clinical immunology.* (2020) 217:108480. doi: 10.1016/j.clim.2020.108480
10. Goriely S, Goldman M. From tolerance to autoimmunity: is there a risk in early life vaccination? *J Comp Pathol.* (2007) 137(Suppl.1):S57–61. doi: 10.1016/j.jcpa.2007.04.013
11. Segal Y, Shoenfeld Y. Vaccine-induced autoimmunity: the role of molecular mimicry and immune crossreaction. *Cell Mol Immunol.* (2018) 15:586–94. doi: 10.1038/cmi.2017.151

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CASE REPORT
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Case Report: Identification of Compound Heterozygous Mutations in a Patient With Late-Onset Glycogen Storage Disease Type II (Pompe Disease)

Huiting Zhang†, Jun Chen†, Yuchang Zhu, Xiaotang Ma and Wangtao Zhong**

Guangdong Key Laboratory of Age-Related Cardiac and Cerebral Diseases, Department of Neurology, Institute of Neurology, Affiliated Hospital of Guangdong Medical University, Zhanjiang, China

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Edited by:
Giovanni Meola, University of Milan, Italy

Reviewed by:
Anna Rubegni, Stella Maris Foundation (IRCCS), Italy
Daria Diodato, Bambino Gesù Children's Hospital (IRCCS), Italy

***Correspondence:**
Xiaotang Ma
mxtgdmc@163.com
Wangtao Zhong
zhongwangtao512@aliyun.com

†These authors have contributed equally to this work and share first authorship

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Pompe disease is an autosomal recessive hereditary lysosomal disorder and correlated with acid α-glucosidase enzyme (GAA) deficiencies, which lead to accumulation of glycogen in all tissues, most notably in skeletal muscles. Adult late-onset Pompe disease (LOPD) is a slowly progressive disease of proximal myopathy with later involvement of the respiratory muscles, resulting in respiratory failure. In this study, we reported a 22-year-old Chinese woman with inability to withstand heavy physical activity since childhood, who presented with respiratory and ambulation weakness in 2 months. On admission, her bilateral upper limbs strength was 4/5 and lower limbs strength was 3/5 according to Medical Research Council (MRC) score. The patient had compound heterozygotes containing a newly identified 4 nt deletion of coding sequence (deletion nt 1411_1414) in one of the acid α-glucosidase alleles and a c.2238G>C (p.Trp746Cys) missense mutation. This deletion has been reported in infant-onset Pompe disease (IOPD) but not LOPD. Intriguingly, this deletion mutation was not found in the patient's family and was considered as pathogenic. Muscle biopsy showed scattered vacuoles with basophilic granules inside the subsarcolemmal area, which were strongly stained by periodic acid-Schiff (PAS). Laboratory tests revealed a significant increase of creatine kinase MB isoenzyme (CK-MB) and lactate dehydrogenase (LDH). GAA level was 9.77 nmol/1 h/mg and was not sufficient for the diagnosis of GAA activity deficiency (0–3.78 nmol/1 h/mg). In summary, mutational analysis of GAA and muscle biopsy are crucial in the diagnosis of Pompe disease.

Keywords: late-onset Pompe disease, glycogen storage disease type II, c.1411_1414del, acid α-glucosidase enzyme, metabolic myopathy

INTRODUCTION

Pompe disease is an inherited metabolic myopathy (1). It is reported that the frequency of Pompe disease is 1:50,000 in China and 1:40,000 in Caucasian populations (1, 2). Considering its defects in acid α-glucosidase enzyme (GAA) activity, which leads to glycogen accumulation in lysosomes, Pompe disease is also known as glycogen storage disease type II (1).

The diagnosis of Pompe disease could be very difficult since its clinical manifestation is highly variable and routine laboratory tests lack sensitivity. Muscle biopsy, GAA activity test, and genetic analysis of the GAA gene play important roles in the diagnosis of Pompe disease. GAA gene is located on chromosome 17q25.2-q25.3 and contains 20 exons. At present, ∼562 mutations have been discovered in GAA (http://www.pompevariantdatabase.nl.) (3). Among them, c.-32-13T>G mutation is common among Caucasian late-onset Pompe disease (LOPD) patients, while it is not found in Asian population (4), and p.R854X mutation is specifically pronounced in African Americans (5). Two variations, p.G576S and p.E689K, are more frequent among Asian population, including the population in Taiwan and Japan (6). These suggest that GAA gene mutation in Pompe disease has regional and ethnic differences. Pompe disease is an autosomal recessive disorder and mainly divided into infant-onset Pompe disease (IOPD) and LOPD. IOPD usually leads to hypertrophic cardiomyopathy. In IOPD, symptoms occur very early (at a median age of 2 months), and death happens soon afterward if the disease remains untreated (by a median age of 8.7 months) (7). As for LOPD, its manifestations are diverse and typically present with ambulatory and respiratory weakness (8). The diagnosis of LOPD is challenging due to very mild clinical presentations or clinical similarities with other muscular diseases. Therefore, GAA sequencing analysis may help screen Pompe disease. In this study, we reported a deletion mutation in GAA gene, which has not been discovered in LOPD.

CASE REPORT

A 22-year-old Chinese woman was referred to our hospital (Affiliated hospital of Guangdong Medical University, Zhanjiang, Guangdong, China), with progressive dyspnea and ambulation weakness for 2 months, especially with difficulties in walking up and down the stairs. She was first treated in the department of respiratory medicine and then referred to the neurological department for further diagnosis and treatment. Her exercise tolerance had been waning since elementary school. She could take care of her daily activities, but could not bear heavy physical work. Her family history of consanguinity was negative and birth history was unremarkable. Both her parents and her sister were workers and were healthy. She was the youngest of three siblings.

On admission at the neurological department, the patient's height and weight were 156 cm and 36.2 kg, respectively. Physical examination revealed that her chest expansion was poor and tendon reflexes were evidently decreased in both upper and lower extremities. Her speaking and swallowing functions were normal and no muscle atrophy was observed. Six groups of muscles (shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension, and foot dorsiflexion) of the patient were assessed by Medical Research Council (MRC) score, which scores muscle strength from 0 to 5. The patient's flexor and extensor muscles of wrist and forearm and intrinsic muscles of feet were scored 5/5. Elbow flexion, wrist and finger extension, foot dorsiflexion, and grip strength were normal. Shoulder abduction strength was 4/5, and hip flexion and knee extension strength were 3/5.

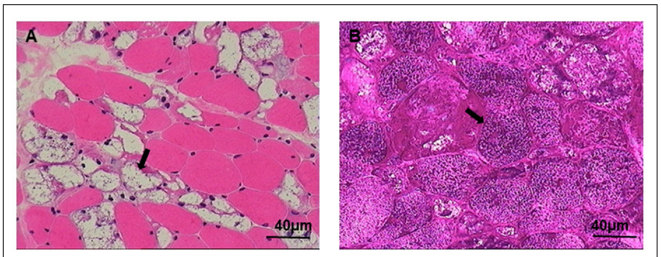

FIGURE 1 | Muscle biopsy is shown using the Hematoxylin and Eosin (HE) and periodic acid-Schiff (PAS) staining. **(A)** Large numbers of glycogen-containing vacuoles were found in the muscle fibers (arrow). **(B)** Vacuoles were PAS positive stained (arrow), scale bar: 40 μm.

Laboratory studies revealed elevated levels of creatine kinase MB isoenzyme (CK-MB) of 51.4 IU/L (normal range: 2.0–5.0 IU/L) and lactate dehydrogenase (LDH) of 392.9 U/L (normal range: 89–221 U/L). Creatine kinase, alanine aminotransferase, and aspartate aminotransferase levels were normal. Red blood cell count (RBC) and hemoglobin (HGB) increased to 7.37×10^{12}/L (normal range: 4.0–5.5/L) and 174.3 g/L (normal range: 110–150 g/L), respectively. Hematocrit (HCT) was also upregulated to 59.8% (normal range: 33.5–45.5%). N-terminal pro-brain natriuretic peptide (NT-proBNP) was 2,038 pg/ml (normal range: 0–300 pg/ml). Rheumatoid factor, C reactive protein, anti-streptolysin O antibodies, erythrocyte sedimentation rate, antinuclear antibody series and antineutrophil cytoplasmic antibody were all negative. Sinus tachycardia, QRS wave right axis deviation, and chest lead clockwise rotation were found by Electrocardiogram (ECG). Echocardiogram and cerebrospinal fluid test were normal.

On the first night of hospitalization in the neurological department, the patient's dyspnea symptom worsened and no dry or wet rales were heard in both lungs. Her heart rate and blood oxygen saturation were 117 beats/min and 97%, respectively. There was no obvious improvement in her symptoms after antiasthmatic, diuretic, and cardiotonic therapy. Additionally, she presented with blurred consciousness, restlessness, and urinary incontinence in short time. Urgent head CT scan showed that multiple overdue strip high-density shadows presented in the sulci of the bilateral cerebral hemispheres, which probably were sulcal blood vessels or subarachnoid hemorrhage (**Supplementary Figure 1**). Chest CT revealed bilateral pleural effusion and inflammation in the lungs, with more significance on the right (**Supplementary Figure 2**). Chest X-ray showed an increased cardiothoracic ratio and inflammation in the lungs. Blood gas analysis showed significantly decreased pH value of 7.057 (normal range: 7.35–7.45) and increased level of pCO_2 of 18.4 kPa (normal range: 4.26–5.99 kPa). Therefore, the patient needed prolonged ventilator support due to decreased inspiratory muscle strength and worsening of lung function.

GAA activity of the patient markedly decreased to 9.77 nmol/1 h/mg (normal >14 nmol/1 h/mg), but was not sufficient for the diagnosis of GAA activity deficiency according to the manufacturer instruction (0–3.78 nmol/1 h/mg). We

FIGURE 2 | Molecular DNA analysis of the patient. **(A,B)** Heterozygous mutations at c.1411_1414del (p.Glu471ProfsTer5) and c.2238G>C (p.Trp746Cys) were detected in the patient.

next performed a muscle biopsy of the left quadriceps, after obtaining written consent from the patient. Results showed that plenty of vacuoles were found in the muscle fiber and muscle pulp, and most vacuoles were in the subsarcolemmal area. Basophilic amorphous materials were detected in the scattered intracytoplasmic vacuoles (**Figure 1A**). Periodic acid-Schiff (PAS) staining disclosed that abnormal glycogen particle deposition, which stained purplish red, were observed in the vacuoles (**Figure 1B**). Genetic analysis revealed two compound heterozygous mutations at c.1411_1414del (p.Glu471ProfsTer5) in exon 9 and c.2238G>C (p.Trp746Cys) in exon16 (**Figures 2A,B**) in the patient. The patient's father had two compound heterozygous mutations for c.1726G>A (p.Gly576Ser) and c.2065G>A (p.Glu689Lys) (**Figure 3A**).

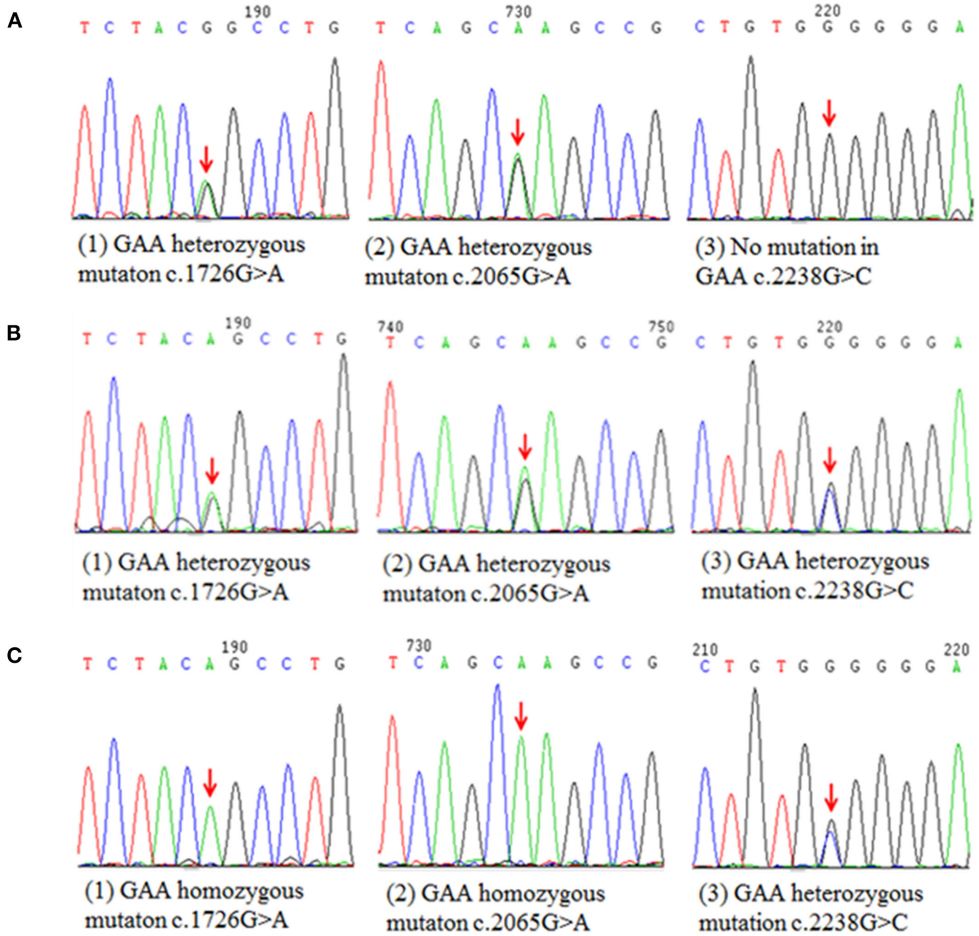

FIGURE 3 | Schematic diagram of the mutation sites of patient's parents and elder sister. **(A)** Two compound heterozygous mutations detected in patient's farther for c.1726G>A (p.Gly576Ser) and c.2065G>A (p.Glu689Lys). **(B)** The patient's mother was heterozygous for c.1726G>A (p.Gly576Ser), c.2065G>A (p.Glu689Lys), and c.2238G>C (p.Trp746Cys) mutations. **(C)** The patient's elder sister was homozygous for c.1726G>A (p.Gly576Ser) and c.2065G>A (p.Glu689Lys) mutations and heterozygous for c.2238G>C (p.Trp746Cys) mutation.

The patient's mother was heterozygous for c.1726G>A (p.Gly576Ser), c.2065G>A (p.Glu689Lys), and c.2238G>C (p.Trp746Cys) mutations (**Figure 3B**), and the patient's elder sister was homozygous for c.1726G>A (p.Gly576Ser) and c.2065G>A (p.Glu689Lys) mutations and heterozygous for c.2238G>C (p.Trp746Cys) mutation (**Figure 3C**). Nerve conduction, electromyography, and muscle MRI examinations were not performed on the patient as she could not be taken off the ventilator.

The patient showed slight improvement in limb weakness after 1 mg neostigmine intramuscular injection. She was first diagnosed with myasthenia gravis and received Gamma globulin (0.4 g/kg × 5 days) combined with methylprednisolone (500 mg/d × 3 days) treatments. However, there was no improvement in her condition, and later the negative results of acetylcholine receptor (AChR) and muscle-specific tyrosine kinase (MuSK) antibodies in serum helped exclude myasthenia gravis. Therefore, the pyridostigmine bromide and corticosteroids treatments were interrupted. The patient continued to receive anti-inflammatory and mechanical ventilation therapy. She had no access to the recombinant human GAA (rhGAA) treatment because of her poor economic condition. A permanent tracheostomy was performed considering her long-term need of ventilator. Besides, physiotherapy and rehabilitation support were implemented. She was discharged with home ventilator support. Her shoulder abduction strength improved to 5/5 and hip flexion and knee extension strength improved to 4/5 on MRC, and the reflexes and ECG were normal. Six months later, the patient could walk without ventilator support for about 15 min and could get rid of ventilator at rest during daytime, as well as while dressing and showering by herself.

DISCUSSION

In this study, we reported a patient who presented with adult-onset respiratory and proximal limbs weakness, and compound heterozygous mutation of the GAA gene. Among the heterozygous mutation, c.1411_1414del (p.Glu471ProfsTer5) was a rare and damaging mutation for LOPD. This study expands the clinical and molecular spectrum of LOPD.

Pompe disease, also known as glycogen storage disease type II or acid maltase deficiency, is an infrequent disorder of the glycogen metabolism (1). The diagnosis of Pompe disease could be relatively simple due to the marked severity of clinical symptoms, muscle pathology, and gene mutation analysis. However, diagnosis of LOPD can be very tough because patients may manifest a more heterogeneous phenotype overlapping with other neuromuscular diseases (9). Adults with LOPD typically present with ambulatory and respiratory difficulties, as in the case that we reported (1). At first, without knowing the results of blood gas analysis and the cause of breath shortness, the patient was treated with medium-high flow oxygen. This oxygen concentration could inhibit the excitatory effect of hypoxia-stimulated respiratory center, resulting in the respiratory depression and failure. We applied myasthenia gravis therapy to treat the patient, but failed to improve her condition. The patient's diagnosis was not confirmed until the results of muscle biopsy and GAA genetic tests were available. Owing to weakness of the respiratory and skeletal muscles caused by Pompe disease, the patient progressively developed chronic hypoxemia and type 2 respiratory failure. Therefore, the patient had to depend on a ventilator. The high levels of RBC, HGB, and HCT might be closely related to the chronic hypoxemia. Chronic hypoxemia could also cause pulmonary vasoconstriction and pulmonary hypertension, and further lead to pulmonary heart disease and right heart failure (HF). The patient presented with more obvious right pleural effusion, which probably resulted from right HF. A study pointed out that the increase in systemic venous pressures causing right HF could prevent venous lymphatic drainage or increase hydrostatic pressure in the bronchial veins and the chest wall, eventually resulting in pleural effusion (10). The increased serum levels of CK-MB and LDH indicated cardiac injury in the patient. The suspicious subarachnoid hemorrhage in the patient's brain was a rare but crucial character of Pompe disease. Excessive glycogen accumulation in the smooth muscle cells of the arteries reduces the elasticity and integrity of the vessel walls, making the brain prone to aneurysms, and intraparenchymal hemorrhage (11). Of note, normal GAA activity should not rule out Pompe disease (12), just as in the case that we reported. Sometimes characteristic muscle histopathology findings of lysosomal glycogenosis and autophagic vacuoles may be totally negative, which makes Pompe disease more difficult to be diagnosed (13). Thus, early and precise diagnosis like GAA gene analysis is needed.

Pompe disease is an autosomal recessive disorder in humans caused by mutations in the GAA gene (1), but the phenotypic expression of this disease happens only if both the alleles of the GAA gene carry a pathogenic mutation (14). In our case, the patient had two compound heterozygous mutations at c.1411_1414del (p.Glu471ProfsTer5) in exon 9 and c.2238G>C (p.Trp746Cys) in exon16. The deletion of 1411_1414 causes a reading frameshift after codon 471, a premature termination signal 12 nucleotides downstream of the deletion and a truncated protein of 474 amino acids. This truncated protein lacks the catalytic site of acid alpha-D-glucosidase, localized to codons 516–520 (15, 16). As far as we acknowledge, c.1411_1414del heterozygous mutation in GAA gene has only been reported in IOPD (16, 17). The second mutation at c.2238G>C causes a change from non-polar aromatic tryptophan to polar aliphatic cysteine at codon 746 and has been known to affect the enzymatic function of acid. One study pointed out that c.2238G>C is the most common mutation among Chinese patients with LOPD (18). In addition, according to the Pompe disease database on http://www.pompecenter.nl, the effect of c.1411_1414del (p.Glu471ProfsTer5) mutation is very severe, while 2238G>C mutation is potentially mild. In other words, the patient carried two potentially pathogenic c.1411_1414del (p.Glu471ProfsTer5) and c.2238G>C (p.Trp746Cys) mutations, and the mutation of c.1411_1414del (p.Glu471ProfsTer5) was likely a more severe pathogenic mutation.

The patient's families had no symptoms of Pompe disease and all carried c.1726G>A (p.Gly576Ser) and c.2065G>A (p.Glu689Lys) common mutations. These two mutations are pseudodeficiency mutations with a high carrying rate in the population (19). Approximately 3.9% of Asians carry c.1726G>A and c.2065G>A homozygous mutations, which lead to the decreased number and activity of the enzyme without causing any clinical symptom (20, 21). The patient's parents and sister were, respectively, heterozygous and homozygous for these two mutations, and her mother and sister had an extra heterozygous mutation of c.2238G>C (p.Trp746Cys), which is a missense mutation (22). Previous studies have demonstrated that the pseudodeficiency mutation of c.2238G>C, c.1726G>A, and c.2065G>A slightly or hardly contribute to Pompe disease (19, 21). Overall, according to the genetic analysis of the patient and her families, the c.1411_1414del (p.Glu471ProfsTer5) mutation is a *de novo* mutation. We theorized that the heterozygous mutations of c.2238G>C (p.Trp746Cys) and c.1411_1414del (p.Glu471ProfsTer5) contributed to Pompe disease. To our knowledge, it is the first time that this deletion mutation was found in LOPD.

CONCLUSION

In this paper, we reported an LOPD patient who manifested with ambulatory and respiratory difficulties, high plasma levels of CK-MB and LDH, glycogen-containing vacuoles of muscle biopsy, and a rare c.1411_1414del (p.Glu471ProfsTer5) mutation. We emphasized the importance of muscle biopsy and GAA gene analysis in diagnosing respiratory and ambulatory weakness. With the finding of this deletion mutation, the genotypic spectrum of Chinese LOPD patients could be extended. Further investigation and analysis of brain magnetic resonance image (MRI), MR venography, MR angiography (MRA), and muscle MRI could provide more insightful understanding of Pompe disease. Medium-high flow oxygen therapy probably could exacerbate respiratory depression/failure without knowing the results of blood gas analysis and the cause of respiratory weakness.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available due to ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Affiliated Hospital of Guangdong Medical University (Permitted Number: PJ2021-094). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HZ, JC, and WZ: conception and design. JC and YZ: performance of experiments. HZ and WZ: manuscript writing. XM and WZ: review, revision, correction of the manuscript, and study supervision. HZ and JC: data analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.839263/full#supplementary-material

Supplementary Figure 1 | Brain computed tomography (CT) images of the patient. Brain CT scan showed scattered high-density areas in bilateral cerebral sulci (red arrows).

Supplementary Figure 2 | Chest CT images of the patient. Chest CT scan showed bilateral pleural effusion and inflammation in the lungs, which was more obvious in the right lung.

Supplementary Figure 3 | Clinical presentation of the patient. **(A)** The patient relied on mechanical ventilation therapy during hospitalization. **(B,C)** No significant muscle atrophy was seen in the patient and she could depend on home ventilator support on discharge.

REFERENCES

1. Van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet.* (2008) 372:1342–53. doi: 10.1016/S0140-6736(08)61555-X
2. De Groot AS, Desai AK, Lelias S, Miah SMS, Terry FE, Khan S, et al. Immune tolerance-adjusted personalized immunogenicity prediction for Pompe Disease. *Front Immunol.* (2021) 12:636731. doi: 10.3389/fimmu.2021.636731
3. Niño MY, In't Groen SLM, Bergsma AJ, van der Beek NAME, Kroos M, Hoogeveen-Westerveld M, et al. Extension of the Pompe mutation database by linking disease-associated variants to clinical severity. *Hum Mutat.* (2019) 40:1954–67. doi: 10.1002/humu.23854
4. Er TK, Chen CC, Chien YH, Liang WC, Kan TM, Jong YJ. Development of a feasible assay for the detection of GAA mutations in patients with Pompe disease. *Clin Chim Acta.* (2014) 429:18–25. doi: 10.1016/j.cca.2013.10.013
5. Cheng YS, Li R, Baskfield A, Beers J, Zou J, Liu C, et al. A human induced pluripotent stem cell line (TRNDi007-B) from an infantile onset Pompe patient carrying p.R854X mutation in the GAA gene. *Stem Cell Res.* (2019) 37:101435. doi: 10.1016/j.scr.2019.101435
6. Kroos MA, Mullaart RA, Van Vliet L, Pomponio RJ, Amartino H, Kolodny EH, et al. p.[G576S; E689K]: pathogenic combination or polymorphism in Pompe disease? *Eur J Hum Genet.* (2008) 16:875–9. doi: 10.1038/ejhg.2008.34
7. Kishnani PS, Hwu WL, Mandel H, Nicolino M, Yong F, Corzo D. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr.* (2006) 148:671–6. doi: 10.1016/j.jpeds.2005.11.033
8. Moriggi M, Capitanio D, Torretta E, Barbacini P, Bragato C, Sartori P, et al. Muscle proteomic profile before and after enzyme replacement therapy in late-onset Pompe Disease. *Int J Mol Sci.* (2021) 22:2850. doi: 10.3390/ijms22062850
9. Jones HN, Hobson-Webb LD, Kuchibhatla M, Crisp KD, Whyte-Rayson A, Batten MT, et al. Tongue weakness and atrophy differentiates late-onset Pompe disease from other forms of acquired/hereditary myopathy. *Mol Genet Metab.* (2021) 133:261–8. doi: 10.1016/j.ymgme.2021.05.005
10. Ferreiro L, Álvarez-Dobaño JM, Valdés L. Can right heart failure cause pleural effusion? *Arch Bronconeumol.* (2019) 55:453–4. doi: 10.1016/j.arbr.2019.02.008
11. Mormina E, Musumeci O, Tessitore A, Ciranni A, Tavilla G, Pitrone A, et al. Intracranial aneurysm management in patients with late-onset Pompe disease (LOPD). *Neurol Sci.* (2021) 42:2411–9. doi: 10.1007/s10072-020-04819-2
12. Kishnani PS, Steiner RD, Bali D, Berger K, Byrne BJ, Case LE, et al. Pompe disease diagnosis and management guideline. *Genet Med.* (2006) 8:267–88. doi: 10.1097/01.gim.0000218152.87434.f3
13. Chan J, Desai AK, Kazi ZB, Corey K, Austin S, Hobson-Webb LD, et al. The emerging phenotype of late-onset Pompe disease: a systematic literature review. *Mol Genet Metab.* (2017) 120:163–72. doi: 10.1016/j.ymgme.2016.12.004
14. Muraoka T, Murao K, Imachi H, Kikuchi F, Yoshimoto T, Iwama H, et al. Novel mutations in the gene encoding acid α-1,4-glucosidase in a patient with late-onset glycogen storage disease type II (Pompe disease) with impaired intelligence. *Intern Med.* (2011) 50:2987–91. doi: 10.2169/internalmedicine.50.5563
15. Hoefsloot LH, Hoogeveen-Westerveld M, Kroos MA, van Beeumen J, Reuser AJ, Oostra BA. Primary structure and processing of lysosomal alpha-glucosidase; homology with the intestinal sucrase-isomaltase complex. *EMBO J.* (1988) 7:1697–704. doi: 10.1002/j.1460-2075.1988.tb02998.x
16. Shieh JJ, Lin CY. Identification of a small deletion in one allele of patients with infantile form of glycogen storage disease type II. *Biochem Biophys Res Commun.* (1996) 219:322–6. doi: 10.1006/bbrc.1996.0231
17. Wan L, Lee CC, Hsu CM, Hwu WL, Yang CC, Tsai CH, et al. Identification of eight novel mutations of the acid alpha-glucosidase gene causing the infantile or juvenile form of glycogen storage disease type II. *J Neurol.* (2008) 255:831–8. doi: 10.1007/s00415-008-0714-0
18. Zhao Y, Wang Z, Lu J, Gu X, Huang Y, Qiu Z, et al. Characteristics of Pompe disease in China: a report from the Pompe registry. *Orphanet J Rare Dis.* (2019) 14:78. doi: 10.1186/s13023-019-1054-0
19. Tajima Y, Matsuzawa F, Aikawa S, Okumiya T, Yoshimizu M, Tsukimura T, et al. Structural and biochemical studies on Pompe disease and a "pseudodeficiency of acid alpha-glucosidase". *J Hum Genet.* (2007) 52:898–906. doi: 10.1007/s10038-007-0191-9
20. Taverna S, Cammarata G, Colomba P, Sciarrino S, Zizzo C, Francofonte D, et al. Pompe disease: pathogenesis, molecular genetics and diagnosis. *Aging.* (2020) 12:15856–74. doi: 10.18632/aging.103794

21. Kumamoto S, Katafuchi T, Nakamura K, Endo F, Oda E, Okuyama T, et al. High frequency of acid alpha-glucosidase pseudodeficiency complicates newborn screening for glycogen storage disease type II in the Japanese population. *Mol Genet Metab.* (2009) 9:190–5. doi: 10.1016/j.ymgme.2009.03.004
22. Jia X, Shao L, Liu C, Chen T, Peng L, Cao Y, et al. GAA compound heterozygous mutations associated with autophagic impairment cause cerebral infarction in Pompe disease. *Aging.* (2020) 12:4268–82. doi: 10.18632/aging.102879

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EDITED BY
Xin-Ming Shen,
Mayo Clinic, United States

REVIEWED BY
Jean-François Desaphy,
University of Bari Aldo Moro, Italy
Karen Joan Suetterlin,
University College London,
United Kingdom
Sophie Nicole,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

*CORRESPONDENCE
Sabrina Lucchiari
sabrina.lucchiari@unimi.it

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Case report: Sodium and chloride muscle channelopathy coexistence: A complicated phenotype and a challenging diagnosis

Serena Pagliarani[1], Giovanni Meola[2,3], Melania Filareti[3], Giacomo Pietro Comi[1] and Sabrina Lucchiari[1]*

[1]Department of Neurological Sciences, Dino Ferrari Centre, IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy, [2]Department of Biomedical Sciences for Health, University of Milano, Milan, Italy, [3]Department of Neurorehabilitation Sciences Casa di Cura del Policlinico, Milan, Italy

Non-dystrophic myotonias (NDM) encompass chloride and sodium channelopathy. Mutations in *CLCN1* lead to either the autosomal dominant form or the recessive form of myotonia congenita (MC). The main symptom is stiffness worsening after rest and improving by physical exercise. Patients with recessive mutations often show muscle hypertrophy, and transient weakness mostly in their lower limbs. Mutations in *SCN4A* can lead to Hyper-, Hypo- or Normo-kalemic Periodic Paralysis or to different forms of myotonia (Paramyotonia Congenita-PMC and Sodium Channel Myotonia-SCM and severe neonatal episodic laryngospasm-SNEL). SCM often presents facial muscle stiffness, cold sensitivity, and muscle pain, whereas myotonia worsens in PMC patients with the repetition of the muscle activity and cold. Patients affected by chloride or sodium channelopathies may show similar phenotypes and symptoms, making the diagnosis more difficult to reach. Herein we present a woman in whom sodium and chloride channelopathies coexist yielding a complex phenotype with features typical of both MC and PMC. Disease onset was in the second decade with asthenia, weakness, warm up and limb stiffness, and her symptoms had been worsening through the years leading to frequent heavy retrosternal compression, tachycardia, stiffness, and symmetrical pain in her lower limbs. She presented severe lid lag myotonia, a hypertrophic appearance at four limbs and myotonic discharges at EMG. Her symptoms have been triggered by exposure to cold and her daily life was impaired. All together, clinical signs and instrumental data led to the hypothesis of PMC and to the administration of mexiletine, then replaced by acetazolamide because of gastrointestinal side effects. Analysis of *SCN4A* revealed a new variant, p.Glu1607del. Nonetheless the severity of myotonia in the lower limbs and her general stiffness led to hypothesize that the

impairment of sodium channel, Nav1.4, alone could not satisfactorily explain the phenotype and a second genetic "factor" was hypothesized. *CLCN1* was targeted, and p.Met485Val was detected in homozygosity. This case highlights that proper identification of signs and symptoms by an expert neurologist is crucial to target a successful genetic diagnosis and appropriate therapy.

KEYWORDS

myotonia, paramyotonia, channelopathies, *CLCN1*, *SCN4A*

Introduction

Muscle chloride and sodium channelopathy are rare non-dystrophic myotonias characterized by myotonia, a prolonged muscle contraction after brief stimuli (a brief excitation) and delayed relaxation following a voluntary contraction.

Mutations in CLC-1, encoded by *CLCN1* gene (RefSeq NC_000007.13), lead to either the autosomal dominant form (Thomsen's disease: OMIM 160800) or the recessive form (Becker's disease: OMIM 255700) of myotonia congenita (MC). Muscle chloride channel works as a homodimer, each dimer representing an ion conductance pathway, the protopore. It is requested for stabilizing the resting membrane potential, and it favors the repolarization of the membrane at the end of depolarization. An impaired channel modifies the cycle of excitability of the myocyte membrane toward hyperexcitability by slowing the return to the resting potential after depolarization. Autosomal dominant myotonia congenita is due to the presence of one dominant-negative mutation that modifies either the gating of both the protopores or the selectivity of one of the two protopores (1, 2). However, some mutations may act as dominant in some patients, and as recessive in others possibly because of incomplete penetrance (3).

The main symptom complained is stiffness worsening after rest and improving by physical exercise (warm up). Patients with recessive mutations often show muscle hypertrophy with different degree and distribution (herculean appearance), and they can suffer from transient weakness at the beginning of voluntary contraction, and this may lead to falls.

Dominant mutations in $Na_v1.4$, encoded by *SCN4A* gene (NC_000017.11) can lead to Hyper-, Hypo- or Normo- kalemic Periodic Paralysis (OMIM 170500) or to different forms of myotonia [Paramyotonia Congenita (PMC): OMIM 168300; Sodium Channel Myotonia (SCM): OMIM 603967]; Severe neonatal episodic laryngospasm (SNEL): OMIM 608390, whereas recessive mutations are associated to congenital myopathy or congenital myasthenic syndromes (OMIM 614198). Sodium channel myotonia is often characterized by facial muscle stiffness, cold sensitivity, and muscle pain. The clinical symptoms are highly variable ranging from a severe neonatal presentation passing through classical SCM to mild, late-onset phenotypes (4). The warm-up phenomenon is usually present in MC patients, but it is sometimes experienced also by SCM patients. On the other hand, PMC patients experience the worsening of myotonia with the repetition of the muscle activity (paradoxical myotonia) and cold, and they could also complain about asthenia and weakness.

Often patients affected by chloride or sodium channelopathies show similar phenotypes and common clinical symptoms, making the diagnosis more difficult to reach.

Herein we present a patient affected by non-dystrophic myotonia where sodium and chloride channelopathies coexist yielding a complex phenotype with features typical of both MC and PMC. The patient's DNA harbors a previously described *CLCN1* mutation, p.Met485Val, in homozygosity, and a novel dominant *SCN4A* variation, p.Glu1607del.

It is well-established in the field of the muscle channelopathies that the application of differential EMG protocols comprehensive of exercise tests may help discriminating the causative gene (5). Nevertheless, this case highlights how crucial it is for the correct identification and attribution of clinical signs and symptoms by the expert neurologist in order to properly redirect the genetic testing and reach a correct diagnosis.

Case description

Herein we describe a 53-year-old woman (DOB 1968) born in Sicily from parents that were first-degree cousins. The disease onset was early when she was 14-year-old, with asthenia, weakness, warm up and stiffness at her arms, and legs after physical activity mainly during volleyball. The symptoms worsened when she, with her parents, moved to the North of Italy where temperatures are cooler. At age of 45 she returned to medical attention complaining about a heavy retrosternal compression, sinus tachycardia with normal ECG, tingling and worsening of myalgia: indeed, ergometric test was interrupted for pain at lower limbs. At the age of 47, after her neuromuscular signs had worsened, she underwent an EMG test at the four limbs showing that myotonic discharges were present in all the tested muscles, especially in biceps brachii and biceps femoris bilaterally. No stimulation test according to Fournier protocol

TABLE 1 Proband's clinical features are recapitulated here.

Characteristics	
Age (yrs)	53
Age of onset (yrs)	14
CLCN1 variant	p.Met485Val/p.Met485Val
SCN4A variant	p.Glu1607del/-
EMG	Myotonic discharges
Handgrip myotonia	No
Orbicularis myotonia	Yes
Lid myotonia	Severe
Paramyotonia	Yes
Muscle stiffness	Severe
Weakness	Moderate, fixed
Muscle pain	Severe
Warm up	No
Muscle hypertrophy	Severe
Triggers for myotonia	Cold

was performed. At age 49 the neurological examination showed evident lid lag and grip myotonia, both worsening with repeated contractions (paradoxical myotonia). Slight muscle weakness (MRC grade 4) at flexor neck muscles, abductors, and flexors (MRC grade 4) of her arms, and flexors (MRC grade 4) of the lower limbs was also present. She had no weakness at distal muscles, and she never complained of adynamia nor paralysis episodes. Laboratory investigations, including CK, were unremarkable. The patient referred an overall worsening of her disease through the years, with difficulties in climbing stairs, and impairing of the daily activities, her work as a hotel housekeeper being impacted significantly. Clinical features are summarized in Table 1.

The patient's father, 75 years old, complained only for myotonic symptoms since when he had moved to the North of Italy for work reasons. As his daughter, he showed palpebral paradoxical myotonia, while he never complained about myalgia or limb stiffness. He worked as a laborer. The Patient's 68 years old mother had no complaints at all, and her neurological examination was unremarkable. Both parents, due to mild and the absence of symptoms, refused to undergo EMG, they agreed only on genetic testing.

Diagnostic assessment and treatments

Instrumental examinations made at disease onset were not available. At age 46 echocardiograms detected slight atrioventricular insufficiency. Because of her difficulty in movements, she underwent lumbosacral MRI, which was negative. EMG examinations performed at age 47 showed myotonic discharges at rest in almost all tested muscles. These data together with orbicular myotonia, and the persistence of stiffness after repeated contractions led to the hypothesis of paramyotonia congenita, and to administration of mexiletine 200 mg once daily, interrupted for gastrointestinal side effects, and replaced by acetazolamide 62.5 mg three times daily without improvement of symptoms. When the patient was 48 years old, lacosamide 50 mg twice daily was tempted to obtain a better control of symptoms, but she positively responded only for a short time, after which cramps started over (Table 2). Concurrently a genetic analysis of the entire *SCN4A* gene was performed revealing a new variant, c.4819_4821delGAG harboring the p.Glu1607del (Figures 1A–C), inherited from the patient's father (Figure 1D). In consideration of the severity of myotonia especially at the lower limbs and because of the general stiffness affecting the patient, it was likely that the phenotype could not be satisfactorily explained by impairment of *SCN4A* gene alone, and the involvement of a second genetic "actor" was hypothesized. Thus, *CLCN1* gene was targeted for a further molecular analysis, and the previously reported mutation p.Met485Val was detected in homozygosity (Figure 1D). Since when the patient was 50 years old, a different pharmacological treatment has been successfully attempted based on lamotrigine 150 mg in the morning and 125 in the afternoon, obtaining improvement of both palpebral and grip myotonia, and limb stiffness.

Discussion

This work describes a patient with non-dystrophic myotonia presenting a complex phenotype not clearly referable to the impairment of a single skeletal muscle channel. Since disease onset, the patient showed a severe range of symptoms which led neurologist to hypothesize a "stiff-person syndrome" vs. sodium channelopathy. Indeed, the presence of severe orbicular myotonia, and the absence of warm up oriented toward a sodium channelopathy. Nevertheless, the severity of myotonia especially at lower limbs and the general stiffness were not satisfactorily explained by the diagnosis of PMC based on clinical symptoms and genetic analysis of *SCN4A*, hence the involvement of a second genetic "player," namely *CLCN1* gene, was hypothesized.

Indeed, the sequencing of the muscle chloride channel revealed a homozygous missense mutation previously described. Both parents, as expected, were heterozygous carrier. The parents did not undergo to any instrumental examination. The nucleotide change c.1453A>G (rs146457619; gnomAD 0.04%; ClinVar 280101) in exon 13 of *CLCN1* yields the missense p.Met485Val which has been reported in a number of studies with myotonia congenita, both in homozygous and compound heterozygous state, while it was detected in heterozygote state in unaffected individuals (6–11). Clinical signs related to the presence of the p.Met485Val were reported by Mazon et al. (12) in a homozygous case sharing with our patient myotonia, and weakness after intense exercise. A clinical description of this mutation in heterozygous compound with p.Ser18Thrfs*55 was

TABLE 2 Timeline of the drug treatments administered to the patient and their effects.

Patient's age	Drug	Dose	Side effects	Efficacy
47	Mexiletine	200 mg sid	Gastrointestinal	Imprv. of Symptoms
47	Acetazolamide	62.5 mg tid	-	No
48	Lacosamide	50 mg bid	-	Brief imprv. of symptoms
50	Lamotrigine	150+125 mg	-	Imprv. of palpebral and grip myotonia, limb stiffness

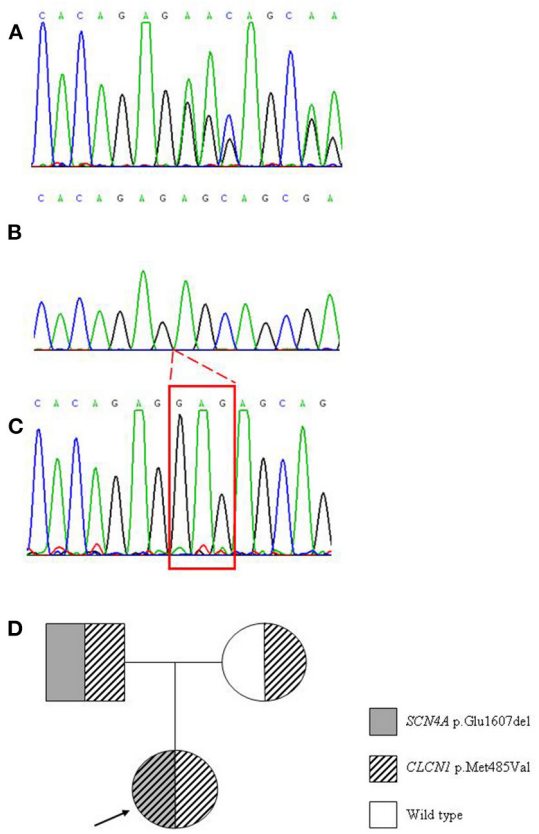

FIGURE 1
Electropherograms from Sanger sequencing showing the patient's altered pattern due to the trinucleotide deletion p.Glu1607del on *SCN4A* gene **(A)** electropherogram obtained after cloning of the PCR product carrying the mutation, in order to resolve the mutated from the wild type allele **(B)** normal pattern in a control case **(C)**. Genetic tree showing the hereditary pattern of both the mutations harbored by the proband (indicated by the arrow) **(D)**.

also done by Hoche et al. (13) in a boy of German/Indian origin presenting with symptoms of severe MC, including stiffness, myotonia after rapid initiation of movements, post-myotonic weakness, muscle pain, lid, percussion, and handgrip myotonia. Functional studies by protein expression in *Xenopus oocytes* had shown that this mutation led to a severe reduction of the single channel conductance becoming strongly inwardly rectifying, compared to wild type, thus the channel was incompletely deactivated at negative voltages (7). In a recent paper by Park and MacKinnon (14) a detailed characterization by Cryo-Electron Microscope of the CLC1 structure was proposed, and the role of Met485 was depicted. This residue is placed above the external chloride binding site located into the protopore, where its flexible side chain would form a constriction near the external end of the ion pathway, and thus would modulate the chloride throughput during membrane repolarization.

The novel *SCN4A* variant c.4819_4821delGAG (p.Glu1607del) was found in heterozygous state in both the proband and her father. It falls in the final part of the transmembrane segment S6 of domain IV of $Na_V1.4$ and is highly conserved among Na_V channels (15) and among species. This variant is not reported in gnomAD, EVS, dbSNP or ClinVar and is predicted to be dangerous by the in silico prediction tool Mutation Taster (Disease causing). The ACMG classification is uncertain significance (PM2, PM4, PP3). To date, only a bunch of in-frame deletions were found on *SCN4A*, and they were all related to sodium channelopathies. The mutation p.Glu36del was described in a patient clinically diagnosed with HypoPP and with a positive LET (long exercise test) (16); p.Lys880del was found in a Japanese patient, and was related to HyperPP (no clinical data) (17), and in a Chinese patient with PMC (no clinical data) (18). The two in-frame deletions of the C-term of $Na_V1.4$ p.Glu1702del and p.Thr1700_Glu1703del were found in myotonic patients and functional studies revealed impairment of fast inactivation for both (19). Thus, although *SCN4A* related channelopathies are mostly caused by missense mutations, there is increasing evidence that little in-frame deletions may play a role. Double trouble cases carrying mutations in both sodium and chloride muscle channels are present in medical literature (20–22). The coexistence of the p.Met485Val with mutations on the *SCN4A* gene has previously been reported by Furby et al. (20) who described a young man harboring p.Gly1306Glu/p.Met485Val, affected from birth, and sharing eye lid myotonia, abundant myotonic discharges in his legs, muscle hypertrophy normalized with age, stiffness, and myalgia with the case studied herein. He had been firstly diagnosed as a case of sodium channelopathy; still genetic findings were not consistent with the type II SET (Short Exercise Test). Sequencing of *CLCN1* found a heterozygous p.Met485 Val.

Our patient was a clinical challenge for several reasons: [1] she presented an atypical course of the disease with an age of onset at 14 years, too late in comparison to a pure sodium channelopathy where symptoms are generally present since infancy. Indeed, it fits more with a chloride channelopathy where symptoms are typically late onset from the second decade; [2] the patient was misdiagnosed for many years with a diagnosis ranging from a demyelinating disorder to stiff-person syndrome to psychiatric tracts. Only the presence of myotonic discharges at EMG correctly oriented toward a skeletal muscle channelopathy. For all these reasons, also a successful pharmacological treatment was hard to reach. First, the patient underwent to mexiletine treatment 200 mg once daily which was stopped for side effects (gastrointestinal), then to acetazolamide 62,5 mg tid, stopped because was ineffective. The next treatment, lacosamide 50 mg twice daily, was administered without effect on myotonia, and later replaced by lamotrigine 150 mg in the morning and 125 mg in the afternoon eliciting positive effects on myotonia (Table 2).

The case described herein highlights that an atypical phenotype—disease onset, a mixture of symptoms and signs—should prompt not to a single channelopathy but to the coexistence of different channel impairment which could explain the complexity of the phenotype. From this perspective, the new variant on *SCN4A* gene, p.Glu1607del, appears as a novel mutation responsible for the PMC phenotype of this case.

Patient perspective

The case described herein emphasizes that the complexity of the mixed phenotypes requires a careful clinical follow-up, and the administration of several drugs throughout the clinical course leading to an improvement in the patient's quality of life.

Written informed consent was provided by the family members involved in this study, for treatment of biological samples, genetic analysis, and sensitive data.

Data availability statement

The dataset in this article are not readily available due to ethical and privacy restrictions. Request to access should be directed to the corresponding author.

Ethics statement

This study was carried out in accordance with the recommendations of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan. All subjects gave written informed consent for genetic analysis in accordance with the Declaration of Helsinki. Written informed consent was obtained from the individual/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

SP and SL contributed to the conceptualization, writing of the paper, methodology, genetic analysis, data collection, and analysis. GM and GC reviewed the paper. GM examined the patients. MF collected the blood and DNA samples. All authors reviewed and approved the paper.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. George AL Jr, Crackower MA, Abdalla JA, Hudson AJ, Ebers GC. Molecular basis of Thomsen's disease (autosomal dominant myotonia congenita). *Nat Genet.* (1993) 3:305–10. doi: 10.1038/ng0493-305

2. Altamura C, Desaphy JF, Conte D, De Luca A, Imbrici P. Skeletal muscle ClC-1 chloride channels in health and diseases. *Pflugers Arch.* (2020) 472:961–75. doi: 10.1007/s00424-020-02376-3

3. Koty PP, Pegoraro E, Hobson G, Marks HG, Turel A, Flagler D, et al. Myotonia and the muscle chloride channel: dominant mutations show variable penetrance and founder effect. *Neurology.* (1996) 47:963–8. doi: 10.1212/wnl.47.4.963

4. Cannon SC. Sodium Channelopathies of Skeletal Muscle. *Handb Exp Pharmacol.* (2018) 246:309–30. doi: 10.1007/164_2017_52

5. Fournier E, Arzel M, Sternberg D, Vicart S, Laforet P, Eymard B, et al. Electromyography guides toward subgroups of mutations in muscle channelopathies. *Ann Neurol.* (2004) 56:650–61. doi: 10.1002/ana.20241

6. Meyer-Kleine C, Steinmeyer K, Ricker K, Jentsch TJ, Koch MC. Spectrum of mutations in the major human skeletal muscle chloride channel gene (CLCN1) leading to myotonia. *Am J Hum Genet.* (1995) 57:1325–34.

7. Wollnik B, Kubisch C, Steinmeyer K, Pusch M. Identification of functionally important regions of the muscular chloride channel CIC-1 by analysis of recessive and dominant myotonic mutations. *Hum Mol Genet.* (1997) 6:805–11. doi: 10.1093/hmg/6.5.805

8. Brugnoni R, Kapetis D, Imbrici P, Pessia M, Canioni E, Colleoni L, et al. A large cohort of myotonia congenita probands: novel mutations and a high-frequency mutation region in exons 4 and 5 of the CLCN1 gene. *J Hum Genet.* (2013) 58:581–7. doi: 10.1038/jhg.2013.58

9. Ferradini V, Cassone M, Nuovo S, Bagni I, D'Apice MR, Botta A, et al. Targeted Next Generation Sequencing in patients with Myotonia Congenita. *Clin Chim Acta.* (2017) 470:1–7. doi: 10.1016/j.cca.2017.04.012

10. Brugnoni R, Maggi L, Canioni E, Verde F, Gallone A, Ariatti A, et al. Next-generation sequencing application to investigate skeletal muscle channelopathies in a large cohort of Italian patients. *Neuromuscul Disord.* (2021) 31:336–47. doi: 10.1016/j.nmd.2020.12.003

11. Dupré N, Chrestian N, Bouchard JP, Rossignol E, Brunet D, Sternberg D, et al. Clinical, electrophysiologic, and genetic study of non-dystrophic myotonia in French-Canadians. *Neuromuscul Disord.* (2009) 19:330–4. doi: 10.1016/j.nmd.2008.01.007

12. Mazón MJ, Barros F, De la Peña P, Quesada JF, Escudero A, Cobo AM, et al. Screening for mutations in Spanish families with myotonia Functional analysis of novel mutations in CLCN1 gene. *Neuromuscul Disord.* (2012) 22:231–43. doi: 10.1016/j.nmd.2011.10.013

13. Hoche F, Seidel K, Barbosa-Sicard E, Heidegger T, Kang JS, Koenig R, et al. Novel N-terminal truncating CLCN1 mutation in severe Becker disease. *Muscle Nerve.* (2014) 50:866–7. doi: 10.1002/mus.24312

14. Park E, MacKinnon R. Structure of the CLC-1 chloride channel from *Homo sapiens*. *Elife.* (2018) 7:e36629. doi: 10.7554/eLife.36629

15. Pan X, Li Z, Zhou Q, Shen H, Wu K, Huang X, et al. Structure of the human voltage-gated sodium channel Nav1.4 in complex with β1. *Science.* (2018) 362:eaau2486. doi: 10.1126/science.aau2486

16. Luo S, Xu M, Sun J, Qiao K, Song J, Cai S, et al. Identification of gene mutations in patients with primary periodic paralysis using targeted next-generation sequencing. *BMC Neurol.* (2019) 19:92. doi: 10.1186/s12883-019-1322-6

17. Sasaki R, Nakaza M, Furuta M, Fujino H, Kubota T, Takahashi MP. Mutation spectrum and health status in skeletal muscle channelopathies in Japan. *Neuromuscul Disord.* (2020) 30:546–53. doi: 10.1016/j.nmd.2020.06.001

18. Yang X, Jia H, An R, Xi J, Xu Y. Sequence CLCN1 and SCN4A in patients with Nondystrophic myotonias in Chinese populations: genetic and pedigree analysis of 10 families and review of the literature. *Channels (Austin).* (2017) 11:55-65. doi: 10.1080/19336950.2016.1212140

19. Horie R, Kubota T, Koh J, Tanaka R, Nakamura Y, Sasaki R, et al. EF hand-like motif mutations of Nav14 C-terminus cause myotonic syndrome by impairing fast inactivation. *Muscle Nerve.* (2020) 61:808–14. doi: 10.1002/mus.26849

20. Furby A, Vicart S, Camdessanché JP, Fournier E, Chabrier S, Lagrue E, et al. Heterozygous CLCN1 mutations can modulate phenotype in sodium channel myotonia. *Neuromuscul Disord.* (2014) 24:953–9. doi: 10.1016/j.nmd.2014.06.439

21. Kato H, Kokunai Y, Dalle C, Kubota T, Madokoro Y, Yuasa H, et al. A case of non-dystrophic myotonia with concomitant mutations in the SCN4A and CLCN1 genes. *J Neurol Sci.* (2016) 369:254–8. doi: 10.1016/j.jns.2016.08.030

22. Maggi L, Ravaglia S, Farinato A, Brugnoni R, Altamura C, Imbrici P, et al. Coexistence of CLCN1 and SCN4A mutations in one family suffering from myotonia. *Neurogenetics.* (2017) 18:219–25. doi: 10.1007/s10048-017-0525-5

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