Received: 22 September 2021 | Revised: 10 March 2022 | Accepted: 15 March 2022

DOI: 10.1002/ccr3.5659

CASE REPORT

Clinical Case Reports Open Access WILEY

Novel compound heterozygous mutations in a GNE myopathy with congenital thrombocytopenia: A case report and literature review

Zhouwei Xu | **Jingyan Xiang** | **Xinghua Luan** | **Zhi Geng** | **Li Cao**

Department of Neurology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

Correspondence
Zhouwei Xu, Department of Neurology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China.
Email: xzwsp@sina.com

Funding information
The research is sponsored by Shanghai Sailing Programme

Abstract

We reported a GNE myopathy with congenital thrombocytopenia on a young male patient. He presented with a 3-year history of lower distal extremity weakness initially affecting his legs. The weakness slowly progressed to lower proximal legs and upper arms last 6 months. Whole-exome sequencing revealed that the patient harbored two heterozygous gene mutations, including a novel insertion mutation c.*1037_*1038CACACACACACACACACACACA and c.C478T in exome 12 and 3 of the GNE gene (NM_001128227), respectively. The levels of serum sialic acid in this patient were considerably decreased. Muscle MRI imaging showed the anterior and medial parts of his quadriceps were heavily affected by this disease. Hematoxylin and eosin staining showed prominent rimmed vacuoles with a lack of inflammatory response in the atrophied muscle. We also undertook a review of the current literature, searching for reports in which the GNE gene mutation caused the thrombocytopenia with or without muscle weakness. This new gene mutation finding broadens the GNE disease genotype spectrum, and further investigation of the relationship between GNE gene mutations and the heterogeneity of its clinical manifestations is needed.

KEYWORDS

compound gene mutations, GNE myopathy, heterogeneity, sialic acid, thrombocytopenia

1 | INTRODUCTION

GNE (OMIM 605820) myopathy is a rare, autosomal recessively inherited myopathy disease caused by mutations in the GNE gene, the gene encoding the bifunctional enzyme UDP-N-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase. This disease is also known as distal myopathy with rimmed vacuoles (DMRV) disease or Nonaka myopathy.[1] The incidence rate of GNE myopathy ranged from 1 to 21 per million.[2,3] The clinical manifestations include distal extremity weakness with early adult-onset, especially foot drop due to the slowly progressive weakness of the anterior tibialis muscle, with quadriceps femoris relatively unaffected. Pathological manifestations of muscle biopsy are prominent rimmed vacuoles without inflammatory response, which can especially be found in atrophied muscle fiber.[4] GNE myopathy has a disease duration ranging from 10 to 20 years.

There is no specific medication for slowing down the progress of this disease. It is not well recognized and increasingly misdiagnosed due to various reasons, including heterogeneous manifestations in the other organ systems and lack of available golden standard diagnostic criteria in the clinical setting. Here, we report a case of GNE myopathy in a young man with congenital thrombocytopenia. The whole-exome sequencing revealed he harbored two compound heterozygous GNE gene mutations (one insertion mutation and one point mutation). Muscle MRI imaging showed the anterior and medial parts of his quadriceps were heavily affected by this disease. We also did muscle biopsy after the patient received the injection treatment of the recombinant human thrombopoietin (rhTPO).

2 | CASE REPORT

A 29-year-old man presented with a 3-year history of lower distal extremity weakness initially affecting his legs. The weakness slowly progressed to lower proximal legs and upper arms last 6 months, and he gradually noticed difficulties climbing up the stairs and lifting the legs and upper arms. He began to have a typical waddling gait with bilateral foot drop 1 month ago. He was detected with idiopathic thrombocytopenia by a local hospital at 2 years old. The platelet count ranged from 10 to 20 $\times$ 10^9/L (reference 125–350 $\times$ 10^9/L) during that period. The patient was treated with prednisolone and vincristine for the next 2 years. The medications for thrombocytopenia were then stopped, and the thrombocytopenia still persisted. The platelet count was maintained from 5 to 15 $\times$ 10^9/L, which induced intermittent nasal bleeding and ecchymosis.

Two years ago, he began to recognize the difficulties in walking and easily fell due to the clubfoot, also known as congenital talipes equino-varus (CTEV). The symptoms worsened over the last 6 months, and the muscle weakness began to affect his upper arm. He noted the difficulties in gripping and holding objects. He was given routine neurotrophic treatment and physiotherapy by a local hospital. After 4 weeks of treatment, the weakness symptom did not improve. The patient was then referred to our hospital for a further neurological consultation.

The physical and neurological examination revealed that he exhibited marked weakness of the bilateral distal and proximal lower limb (Medical Research Council grade 2/5 ankle dorsiflexion and eversion, grade 3/5 ankle plantar flexion and inversion, grade 3/5 hip flexion and abduction). His first interosseous muscle was slightly affected. His bilateral ankle reflexes were absent. There were serious purpura on his back and knee skin. The EMG showed

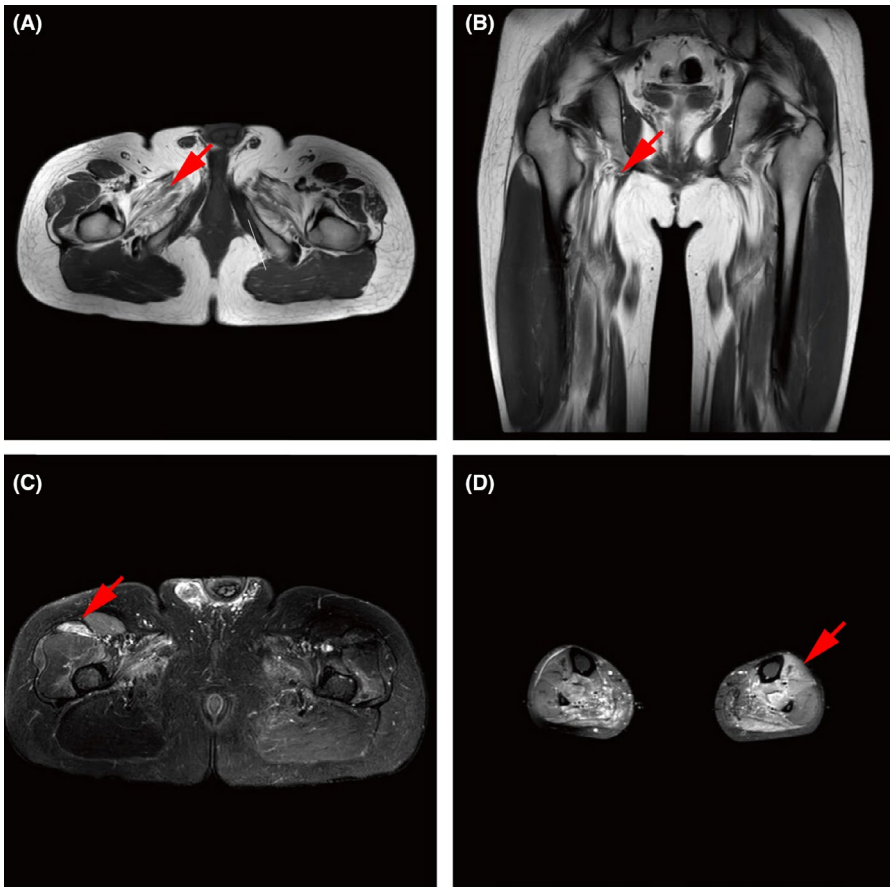

FIGURE 1 (A) Axial T1 weighted image of thigh and buttock muscles. (B) Coronal T1 weighted image of thigh and buttock muscles. (C) Axial STIR image of thigh and buttock muscles. (D) Axial T1 weighted image of lower limbs. MRI images showed extensive fatty replacement in the distal extremity limbs, especially the medial compartment of the legs. The anterior and medial compartment of the thigh and buttock muscles were also heavily involved (red arrows)

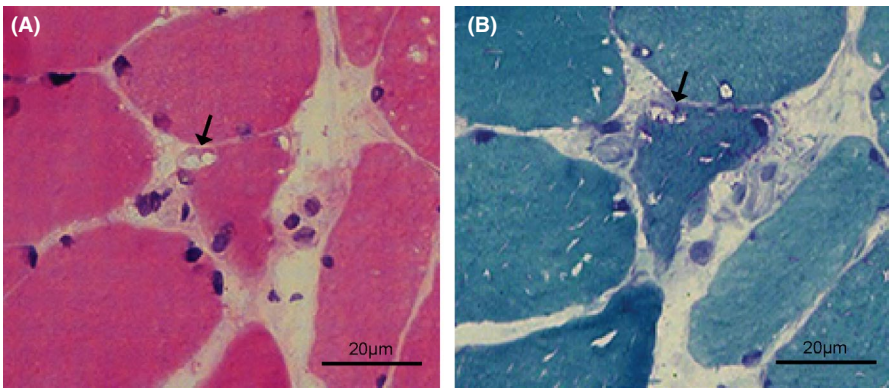

FIGURE 2 Hematoxylin and eosin staining showed prominent rimmed vacuoles with a lack of inflammatory response in the atrophied muscle. Modified Gomori trichorme staining highlighted the vacuoles in several muscle fibers (black arrows)

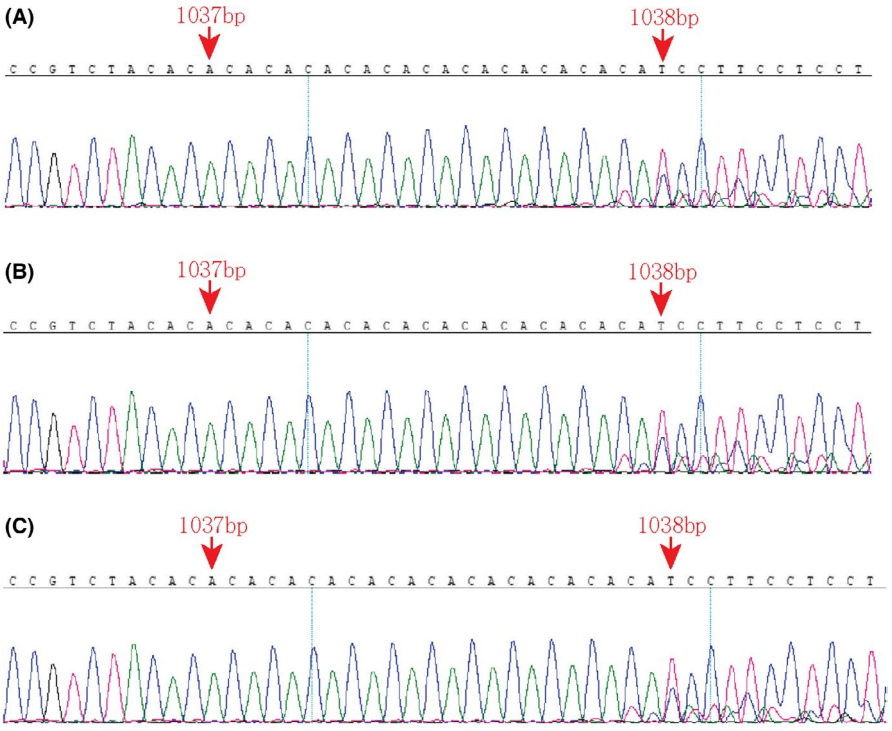

FIGURE 3 c.*1037_*1038CACA CACACACACACACACACA insertion in exome 12 of GNE. (A) Patient (heterozygous). (B) Mother (heterozygous). (C) Father (heterozygous). Red arrows illustrated the 1037 and 1038 bp position in the wild type sequence. The insertion mutation was located between these two positions

myopathic changes in the bilateral legs and neuropathic changes in the left peroneal nerve. Sensory nerve conduction was normal. An additional brain and whole-body muscle MRI scan were performed. The lower limbs MRI scan indicated that there was serious fatty replacement in the distal extremity limbs, especially the medial compartment of the legs (tibialis anterior, hamstring, gastrocnemius). The sartorius, gracilis, and semimembranosus were also seriously replaced by the adipose tissue on T1 sequence and the muscle edema is evident on short TI inversion recovery (STIR) sequence (Figure 1). Brain MRI and other cardiorespiratory examinations were normal. The blood test showed a low platelet count 22×10^9/L. The mean platelet volume is 8 fl (reference 7–11 fl) and the platelet-associated antibody was negative. There were no abnormalities on his other blood test, renal, liver, thyroid function, erythrocyte sedimentation rate, and antinuclear antibody panel. The level of creatine kinase was elevated, with the highest level recorded as 405 U/L (reference 21–190 U/L). The liquid chromatography–mass spectrometry (LC–MS) indicated that the level of very long-chain fatty acid (VLCFA) was within the normal range. The previous history revealed that he had half a year of one small nodule on his left lung. He did not have a history of smoking and any other drug addiction, alcohol overuse, or vaccination. He also had no significant family history of neuromuscular diseases. His parents were nonconsanguineous and had no relevant symptoms. He has no other siblings.

Due to the risk of bleeding, the patient was referred to the hematology department, and a bone marrow aspiration was performed. The results showed that the number of mega-karyocytes increased and the platelets' size was normal, whereas the number of thrombocytogenousmegakaryocyte was decreased. The previous diagnosis of idiopathic thrombocytopenia was confirmed. The patient was then prescribed the recombinant human thrombopoietin

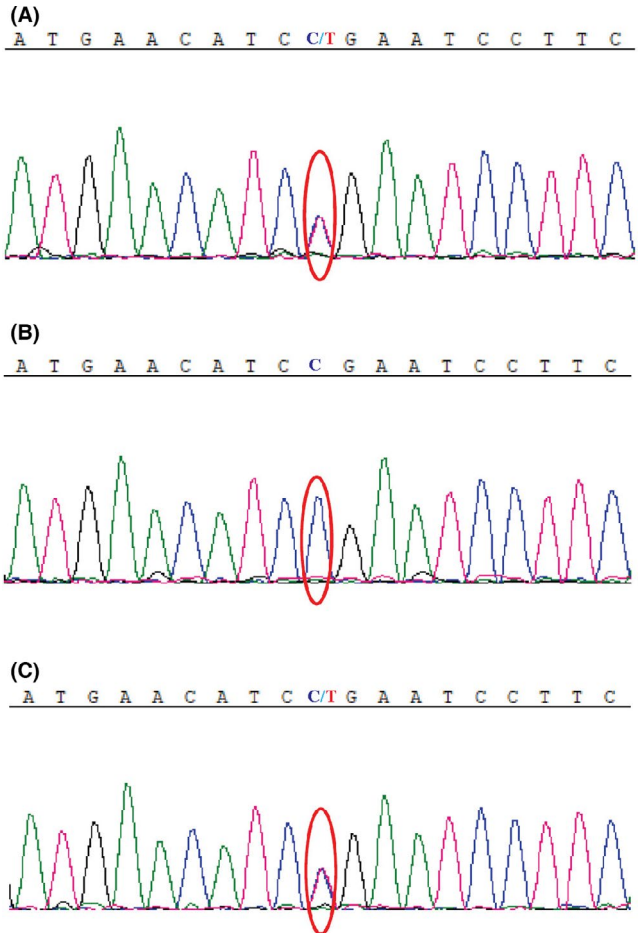

FIGURE 4 c.C478T point mutation in the exome 3. (A) Patient (heterozygous). (B) Mother (wild type). (C) Father (heterozygous)

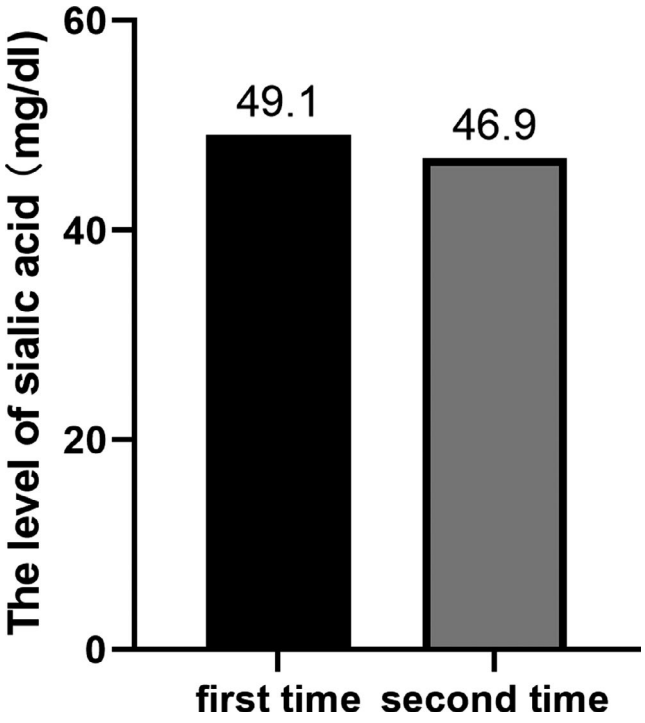

FIGURE 5 Levels of sialic acid in this patient's serum were detected by ELISA twice during his first and second admission, which is much lower than normal range (reference 51–84 mg/dl). The second result is markedly downregulated in comparison with the first one

(rhTPO) to promote platelet generation. He received a subcutaneous injection treatment of rhTPO for the next 2 weeks and was discharged from the hospital.

The patient was admitted to the department of neurology again after 1 month. The number of platelet was elevated to 55×10^9/L. A muscle biopsy on the rectus femoris was performed. Hematoxylin and eosin staining showed prominent rimmed vacuoles with a lack of inflammatory response in the atrophied muscle. Modified Gomori trichorme staining also highlighted the vacuoles in several muscle fibers (Figure 2). NADH-tetrazolium reductase staining showed that the muscle fiber was almost normal.

Whole-exome sequencing revealed that the patient harbored two heterozygous gene mutations, including a novel insertion mutation c.*1037_*1038CACACACACACACACACACACA and c.C478T in exome 12 and 3 of the GNE gene (NM_001128227), respectively. The first mutation was located in the 3′ noncoding regions. The later gene mutation changed the 160th amino acid into stop code, and the final produced protein has 594 shorter amino acids than the normal one with 753 amino acids. A heterozygous variant was identified on his parents for the first gene mutation (Figure 3). His mother and father had a wild type and a heterozygous type for the second mutation, respectively (Figure 4). The c.C478T mutation was predicted to be "damaging" and "disease causing" by in silico analyses implemented using the bioinformatics software Mutation Taser and Sorting Intolerant From Tolerant (SIFT). The levels of serum sialic acid in this patient were measured by using enzyme-linked immunosorbent assay (ELISA) twice during his hospitalization and were markedly below the normal range (Figure 5).

The patient was finally diagnosed with GNE myopathy based on the previous medical history, neurological findings, muscle biopsy, and MRI scan, which fulfilled the diagnostic criteria of GNE myopathy. The patient was empirically treated with oral sialic acid, coenzyme Q10, and physiotherapy.

3 | DISCUSSION

Up to date, nearly 200 mutations have been found in the GNE myopathy.[5] The point c.C478T mutation was previously reported in other studies.[6,7] Interestingly, these cases had only muscle atrophy without concomitant thrombocytopenia. To our knowledge, the c.*1037_*1038CACACACACACACACACACACA mutation

TABLE 1 Review of the published literature (GNE associated with thrombocytopenia)

Author	Sex	Age	Myopathy	Thrombocytopenia (10^9/L)	GNE gene mutation	Other symptoms
Izumi et al.[20]	Male	32	Proximal lower limb	1.7–16.2	c.1807G>C p.V603L c.2215G>A p.G739S	Membrnoproliferative glomerulonephritis
Izumi et al.[20]	Female	29	Neck and overall proximal muscle	1.1–9.0	c.1807G>C p.V603L c.2215G>A p.G739S	Moderate splenomegaly
Paul and Liewluck[18]	Female	50	Distal myopathy	71	c.1900C>T(p.Leu634Phe), c.125G>A(p.Arg42Gln)	Encephalopathy,hemolytic anemia,hemolytic uremic syndrome
Mori-Yoshimura et al.[21]	N/A	N/A	Distal myopathy	7.1–10.3	p.R420X/p.V572L,383insT/p.V572L,p.R8X/p.V572L	NO
Revel Vilk et al.[19]	5 patients (3 females, 2 males)	24–42	NO	1–4	c.1516_1517delinsTT, p.Gly475Phe	NO
Revel Vilk et al.[19]	3 patients (2 females, 1 male)	6–14	NO	3–10	c.1457T>C, p.Leu486Pro	NO
Revel Vilk et al.[19]	Male	11	Hypotonia of the lower extremities	30–40	c.1649A>G, p.Asn550Ser c.562C>T, p.His188Tyr	Autism
Zhen et al.[22]	Female	29	Hands and the distal parts of her legs	3.6	c.649T>C p.T217H c.1543-1544 del GA (p.A515Gfs*2)	NO
Zhen et al.[22]	Male	26	Gastrocnemious and foot muscle	4.5	c.649T>C p.T217H c.1543-1544 del GA (p.A515Gfs*2)	NO
Yilmaz et al.[23]	Male	30 days	NO	6	Chr9:36219976:C>T p.590:Gly>Arg	Neurtropenia
Futterer et al.[24]	Male	3	NO	10	p.G416R	Hydrocephalus, nystagmus, developmental delay and skull abnormality
Futterer et al.[24]	Female	7	NO	15–20	p.G416R	Epistaxis

has not been reported in GNE patients with thrombocytopenia before. Previous study indicated that most of the GNE myopathy patients harbored a homozygous mutation or compound heterozygous mutations.[8] The patient's father was also detected with the same compound mutations. However, no muscle atrophy or relevant clinical manifestations were found on him. The reason could be due to the different genetic backgrounds or epigenetic factors. One study demonstrated that histone methylation could regulate and influence the myogenesis.[9] Currently, there is no clear established genotype–phenotype correlations for GNE myopathy The relationship between the phenotype variation and this novel insertion genotype is still elusive.

The GNE myopathy is characterized by selectively sparing the quadriceps. However, the sparing of quadriceps is not invariably present in this disease. Several studies have indicated that the muscle weakness could progress to the proximal part of the lower limb in the advanced stage of the disease partially due to the high concentration of sialic acid in quadriceps.[4,10] In this case, this patient's quadriceps were heavily affected. There were severe fatty degenerative changes in the proximal part of the lower limb, especially the gracilis and semimembranosus. This pattern of muscle weakness was more profound in the medial part of both the lower and proximal limbs on the MRI imaging. One study showed that the vastus lateralis was the least affected part of the quatriceps on GNE patients.[11] The variation in vulnerability for different muscles could be a clue for the future possible therapy.

Several studies have reported that the thrombocytopenia was closely associated with the GNE gene mutation. We undertook a review of the current literature, searching for reports in which the GNE gene mutation caused the thrombocytopenia with or without muscle weakness. The literatures about the myopathy associated with the GNE mutations and the relevant manifestations were summarized in Table 1. The GNE gene encodes a enzyme that regulates the rate of the biosynthesis of the sialic acid, which plays a key role in the platelet's sialylation and further affects the lifetime of the platelet. Platelet membranes glycoprotein Ib (GP Ib) are known to contain sialic acid, which is involved in the platelet aggregation and adhesion pathways. One study showed that platelet sialic acid cleavage was closely associated with the number of platelet in the circulation.[12] Another study revealed that sialic acid is critical for complement-mediated regulation on the platelets.[13] Using the sialidase inhibitor could elevate the count of the platelet in vivo.[14] Low concentration of sialic acid in this patient's serum was observed and indicated that the biosynthesis of sialic acid was affected. Yonekawa et al. used oral sialic acid supplementation to prevent muscle weakness and atrophy in a GNE mouse model.[15] However, limited benefits were seen in the clinical trials with sialic acid supplement and Lochmüller et al. found that aceneuramic acid extended-release (Ace-ER) did not improve muscle strength and function in patients with GNE myopathy.[16] N-acetyl-D-mannosamine (ManNAc), an uncharged monosaccharide, was found to effectively elevate the level of sialic acid in the blood of the GNE patients and could be a potential therapy candidate.[17] Interestingly, this patient's congenital thrombocytopenia was found prior to the muscle weakness. It is unclear whether the megakaryocytic system is more vulnerable to the different GNE mutation than the skeleton muscle system. And several studies have also shown that the different GNE variants are associated with other organ dysfunction, including the psychological, renal, and vestibular system.[18,19]

In this case, we speculate that the novel insertion mutation contribute to the dysfunction of the GNE enzyme, downregulating the expression level of the sialic acid and further decrease the platelet's number in the blood circulation. The effect of this gene mutation on megakaryocytes and myogenesis remains to be elucidated.

4 | CONCLUSION

In conclusion, we reported a novel heterozygous gene mutation in a young patient. To our knowledge, this is the first GNE myopathy case with insertion mutation (c.*1037_*1038CACACACACACACACACACA) also has thrombocytopenia. The exact mechanism of the sialic acid in the thrombocyte biosynthesis and skeleton muscle is needed to be clarified. The sialic acid supplementation therapy and gene editing therapy for the disease in vivo are promising.

This finding broadens the GNE disease genotype spectrum, and further investigation of the relationship between GNE gene mutations and the heterogeneity of its clinical manifestations is needed.

ACKNOWLEDGMENT

Thank Doctor Yuwu Zhao for giving the advices on gene mutation analysis.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Zhouwei Xu and Jingyan Xiang conceived and designed the study. Xinghua Luan, Zhi Geng and Li Cao involved in overall supervision of the paper. All authors read and approved the final manuscript.

ETHICAL APPROVAL

This study was approved by the ethical committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (2019–008).

CONSENT

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

ORCID

Zhouwei Xu https://orcid.org/0000-0001-5735-9987
Li Cao https://orcid.org/0000-0003-1742-9877

REFERENCES

1. Mori-Yoshimura M, Oya Y, Yajima H, et al. GNE myopathy: a prospective natural history study of disease progression. *Neuromuscul Disord*. 2014;24(5):380-386. doi:10.1016/j.nmd.2014.02.008
2. Carrillo N, Malicdan MC, Huizing M. GNE myopathy: etiology, diagnosis, and therapeutic challenges. *Neurotherapeutics*. 2018;15(4):900-914. doi:10.1007/s13311-018-0671-y
3. Wu Y, Yuan L, Guo Y, et al. Identification of a GNE homozygous mutation in a Han-Chinese family with GNE myopathy. *J Cell Mol Med*. 2018;22(11):5533-5538. doi:10.1111/jcmm.13827
4. Ghaoui R, Palmio J, Brewer J, et al. Mutations in HSPB8 causing a new phenotype of distal myopathy and motor neuropathy. *Neurology*. 2016;86(4):391-398. doi:10.1212/WNL.0000000000002324
5. Grecu N, Villa L, Cavalli M, et al. Motor axonal neuropathy associated with GNE mutations. *Muscle Nerve*. 2020. doi:10.1002/mus.27102
6. Bhattacharya S, Khadilkar SV, Nalini A, et al. Mutation spectrum of GNE myopathy in the Indian sub-continent. *J Neuromuscul Dis*. 2018;5(1):85-92. doi:10.3233/JND-170270
7. Celeste FV, Vilboux T, Ciccone C, et al. Mutation update for GNE gene variants associated with GNE myopathy. *Hum Mutat*. 2014;35(8):915-926. doi:10.1002/humu.22583
8. Mori-Yoshimura M, Hayashi YK, Yonemoto N, et al. Nationwide patient registry for GNE myopathy in Japan. *Orphanet J Rare Dis*. 2014;9:1:150. doi:10.1186/s13023-014-0150-4
9. Jin W, Peng J, Jiang S. The epigenetic regulation of embryonic myogenesis and adult muscle regeneration by histone methylation modification. *Biochem Biophys Rep*. 2016;6:209-219. doi:10.1016/j.bbrep.2016.04.009
10. Chan YM, Lee P, Jungles S, et al. Substantial deficiency of free sialic acid in muscles of patients with GNE myopathy and in a mouse model. *PLoS One*. 2017;12(3):e0173261. doi:10.1371/journal.pone.0173261
11. Park H, Lee J, Na S, Park K, Choi Y. Pattern analysis in Korean patients with distal myopathy using lower-limb MRI. *Neuromuscul Disord*. 2016;26:S101. doi:10.1016/j.nmd.2016.06.059
12. Revilla N, Corral J, Miñano A, et al. Multirefractory primary immune thrombocytopenia; targeting the decreased sialic acid content. *Platelets*. 2019;30(6):743-751. doi:10.1080/09537104.2018.1513476
13. Hyvärinen S, Meri S, Jokiranta TS. Disturbed sialic acid recognition on endothelial cells and platelets in complement attack causes atypical hemolytic uremic syndrome. *Blood*. 2016;127(22):2701-2710. doi:10.1182/blood-2015-11-680009
14. Jansen AJ, Peng J, Zhao HG, Hou M, Ni H. Sialidase inhibition to increase platelet counts: a new treatment option for thrombocytopenia. *Am J Hematol*. 2015;90(5):E94-E95. doi:10.1002/ajh.23953
15. Yonekawa T, Malicdan MCV, Cho A, et al. Sialyllactose ameliorates myopathic phenotypes in symptomatic GNE myopathy model mice. *Brain*. 2014;137(Pt 10):2670-2679. doi:10.1093/brain/awu210
16. Lochmüller H, Behin A, Caraco Y, et al. A phase 3 randomized study evaluating sialic acid extended-release for GNE myopathy. *Neurology*. 2019;92(18):e2109-e2117. doi:10.1212/WNL.0000000000006932
17. Xu X, Wang AQ, Latham LL, et al. Safety, pharmacokinetics and sialic acid production after oral administration of N-acetylmannosamine (ManNAc) to subjects with GNE myopathy. *Mol Genet Metab*. 2017;122(1–2):126-134. doi:10.1016/j.ymgme.2017.04.010
18. Paul P, Liewluck T. Distal myopathy and thrombocytopenia due to a novel GNE mutation. *J Neurol Sci*. 2020;415:116954. doi:10.1016/j.jns.2020.116954
19. Revel-Vilk S, Shai E, Turro E, et al. GNE variants causing autosomal recessive macrothrombocytopenia without associated muscle wasting. *Blood*. 2018;132(17):1851-1854. doi:10.1182/blood-2018-04-845545
20. Izumi R, Niihori T, Suzuki N, et al. GNE myopathy associated with congenital thrombocytopenia: a report of two siblings. *Neuromuscul Disord*. 2014;24(12):1068-1072. doi:10.1016/j.nmd.2014.07.008
21. Mori-Yoshimura M, Hayashi YK, Yonemoto N, et al. Nationwide patient registry for GNE myopathy in Japan. *Orphanet J Rare Dis*. 2014;9:150. doi:10.1186/s13023-014-0150-4
22. Zhen C, Guo F, Fang X, Liu Y, Wang X. A family with distal myopathy with rimmed vacuoles associated with thrombocytopenia. *Neurol Sci*. 2014;35(9):1479-1481. doi:10.1007/s10072-014-1790-9
23. Yilmaz E, Ozcan A, Verboon JM, et al. Pb1860 a Turkish child presented with neonatal thrombocytopenia associated with GNE mutation without associated muscle wasting. *HemaSphere*. 2019;3(S1):848-849.
24. Futterer J, Dalby A, Lowe GC, et al. Mutation in GNE is associated with severe congenital thrombocytopenia. *Blood*. 2018;132(17):1855-1858. doi:10.1182/blood-2018-04-847798

How to cite this article: Xu Z, Xiang J, Luan X, Geng Z, Cao L. Novel compound heterozygous mutations in a GNE myopathy with congenital thrombocytopenia: A case report and literature review. *Clin Case Rep*. 2022;10:e05659. doi:10.1002/ccr3.5659