The analysis of genetic variants in nine patients with Nemaline Myopathy: Implications for diagnosis and genetic counseling

(Supplementary material)

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Supplementary Information 1

Table S1 The clinical information of 9 patients in this study

Patient No.	Gender	Clinical phenotyping
1	Female	A 9-month-old girl presented with delayed development, overall weakness and congenital heart disease. She also has a healthy older brother. The Ultrasonic cardiogram (UCG) scan of the proband revealed ventricular septal defect (VSD) and patent foramen ovale (PFO), specifically demonstrating left-to-right shunts. Meanwhile, this patient also exhibited a slightly enlarged left heart, a small amount of tricuspid regurgitation and mild pulmonary hypertension. Fortunately, both the systolic and diastolic functions of the left and right ventricles were normal.
2	Male	A 1-year-old boy came to the hospital for medical consultation because of delayed growth and development and recurrent pneumonia. The Computed Tomography (CT) scan of the lungs revealed a symmetrical chest structure, with the trachea and mediastinum centered. The texture of both lungs has increased, and there are patchy high-density shadows visible in both lungs, with blurred edges. The left lung is more obvious. The size and shape of the heart shadow remain normal. The bilateral diaphragmatic muscles are smooth, and the rib angles are sharp. Meanwhile, the blood routine test indicates an elevation in white blood cells, a rise in the proportion of lymphocytes, and a reduction in the proportion of neutrophils.
3	Male	A 38-day-old male infant (G1P1) presented with a significant elevation of cardiac enzymes, with Creatine Kinase (CK) at 7,135 U/L and CK-MB at 1,090.5 U/L. To investigate the etiology, family-based whole-exome sequencing (WES) was performed, identifying compound heterozygous variants in the NEB gene. Specifically, the paternal variant (c.9103-4_9103-3delTT) was classified as a Variant of Uncertain Significance (VUS). Following genetic counseling, the family was advised on the potential pathogenicity of the VUS, and a management plan involving regular cardiac monitoring and supportive therapy was initiated. At the 9-month follow-up, the patient's CK and CK-MB levels had decreased to 183 U/L and 58.6 U/L, respectively. Concurrent thyroid function testing revealed abnormal serum free T3 levels, leading to a diagnosis of hypothyroidism. Following the initiation of Levothyroxine Sodium, all biochemical parameters normalized. The patient's growth and developmental milestones are currently within age-appropriate limits.
4	Male	A 2-year-old boy came to the hospital seeking medical advice due to poor mental response and recurrent bronchopneumonia. He presented with bluish-purple lips, poor

		appetite, and occasional cough. The UCG findings indicated partial anomalous pulmonary vein drainage, right heart enlargement, pulmonary arterial hypertension, patent foramen ovale, and central atrial septal defect. The CT scan revealed multiple wind cords in both lungs, and multiple ventilation hypoperfusion in both lungs, which might suggest inflammatory changes. Additionally, the child also exhibited symptoms such as delayed growth and development, muscle weakness, etc.
5	Male	A 14-year-old boy came to the hospital to seek medical advice because of delayed language development, delayed motor development, and scoliosis. He began to speak at approximately the age of 3, started walking independently at approximately the age of 5, and the scoliosis was found at approximately the age of 12. He had a lean body and had special facial features, including a thin and elongated face, an elongated middle, synophrys, protruding ears, and snaggle-tooth. There is an older sister who is currently 15 years old and in good physical condition
6	Female	A 4-year-old girl came to the hospital to seek medical advice because of recurrent bronchopneumonia. The patient developed paroxysmal cough without an obvious cause 6 months before seeking medical treatment. Severe coughing can cause vomiting, accompanied by nasal congestion and runny nose, and with fever while the disease. In addition, according to the parents' account, the child walks unsteadily and is prone to falling.
7	Female	An 8-year-old girl presented with symptoms like an inability to lift her head, neck weakness, low muscle tone in the lower limbs, and an inability to turn over. The MRI and susceptibility weighted imaging disclosed that the myelination process was slightly delayed compared to that of her peers. The results of urine gas chromatography-mass spectrometry detection uncovered ketosis and dicarboxylic aciduria. We assessed the Gesell Developmental Schedules (GDS) scores of the patient, and it was revealed that the developmental quotient was 59.2.
8	Male	A 9-month-old boy whose USG showed patent foramen ovale, atrial septal defect, left to right shunt at the atrial level, mild enlargement of the right heart, slight widening of the main pulmonary artery diameter, mild thickening of the left ventricular wall, a small amount of tricuspid regurgitation, a small amount of pulmonary valve regurgitation, and mild pulmonary hypertension. The preliminary clinical diagnosis is severe pneumonia and growth retardation.
9	Unknown	At a gestational age of 25^{+5} weeks, the ultrasound indicates the existence of a cervical water sac tumor along with slight skin edema throughout the body.

Supplementary Information 2

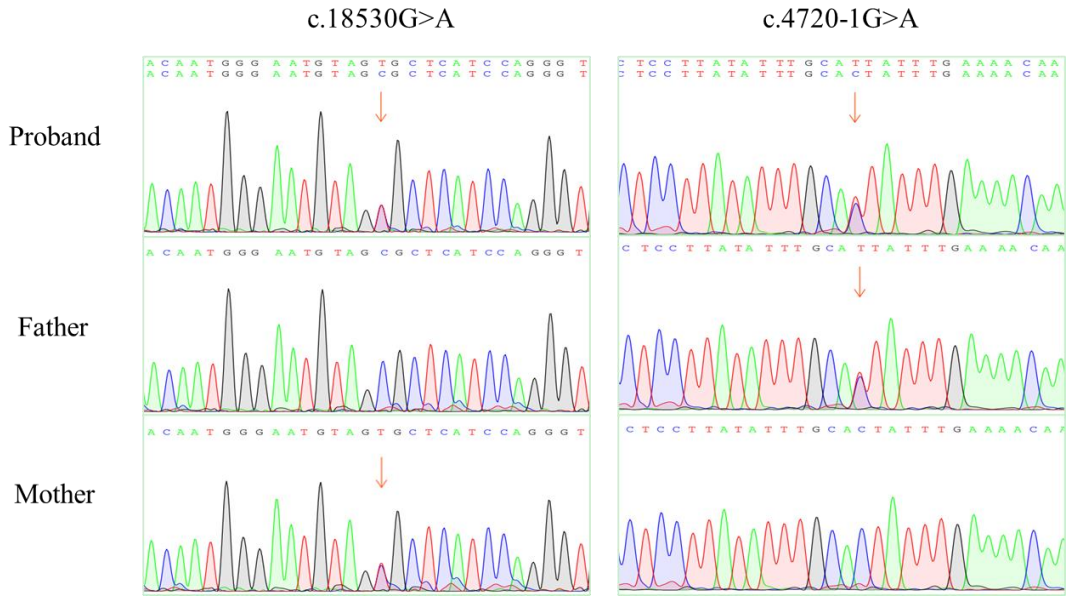

Figure S1 Sanger sequencing results for *NEB* gene variants in Patient 1. The c.18530G>A was inherited from her mother, and the c.4720-1G>A was inherited from her father.

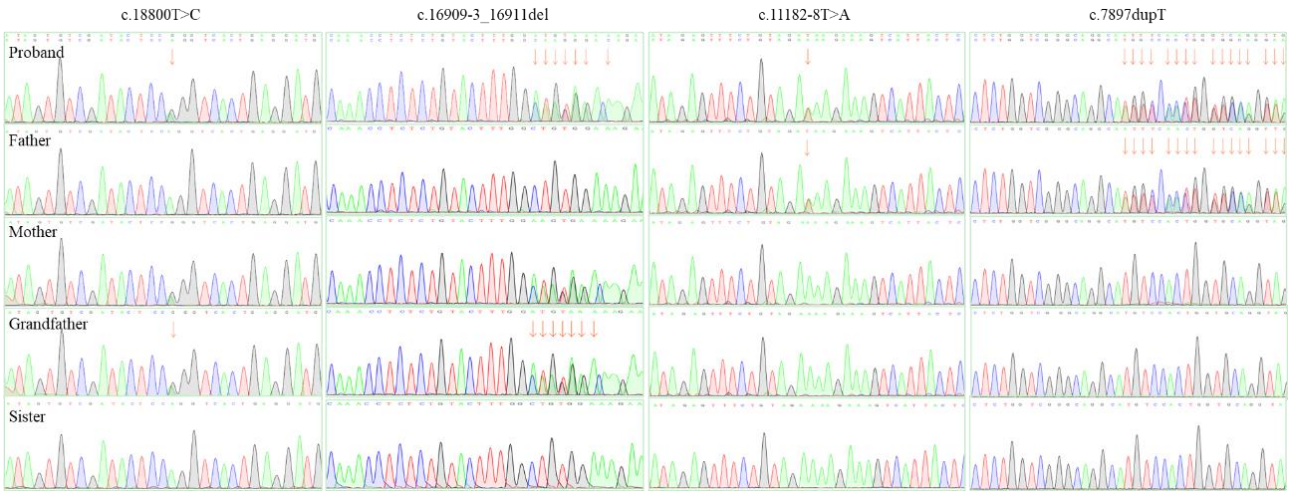

Figure S2 Sanger sequencing results for *NEB* gene variants in Patient 2. The c.18800T>C and c.16909-3_16911del were inherited from her mother and maternal grandfather, and the c.11182-8T>A and c.7897dupT were inherited from her father. The sister these variants are all wild type.

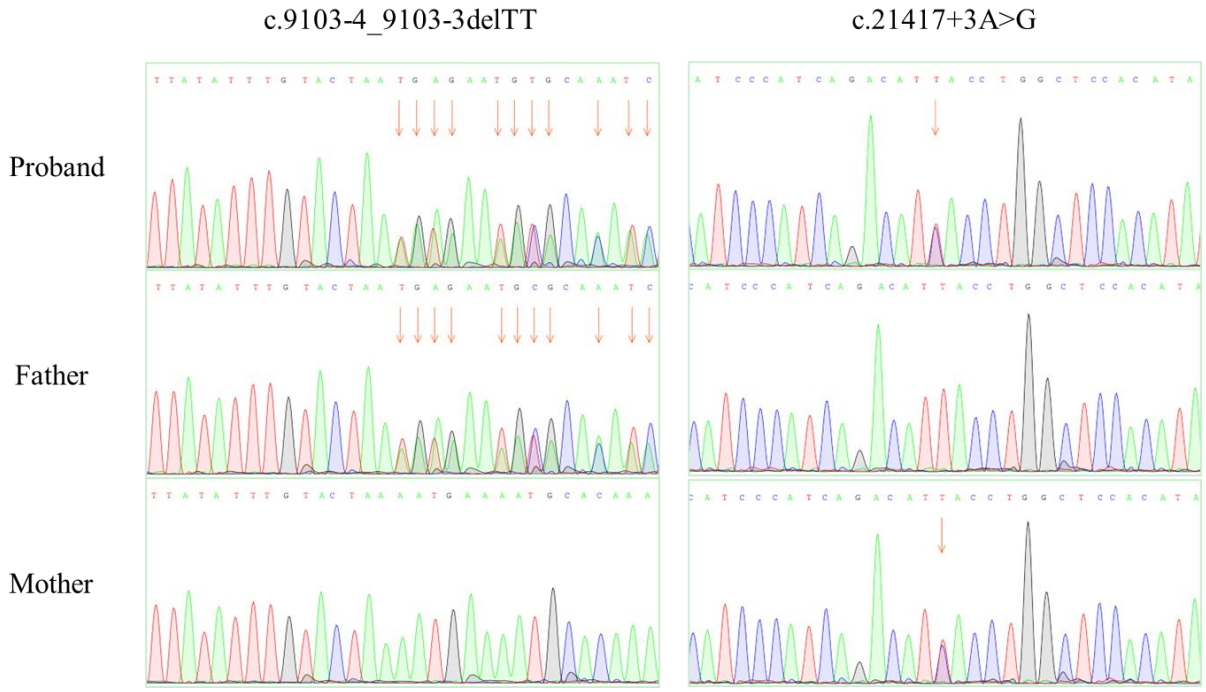

Figure S3 Sanger sequencing results for *NEB* gene variants in Patient 3. The c.21417+3A>G was inherited from her mother, and the c.9103-4_9103-3delTT was inherited from her father.

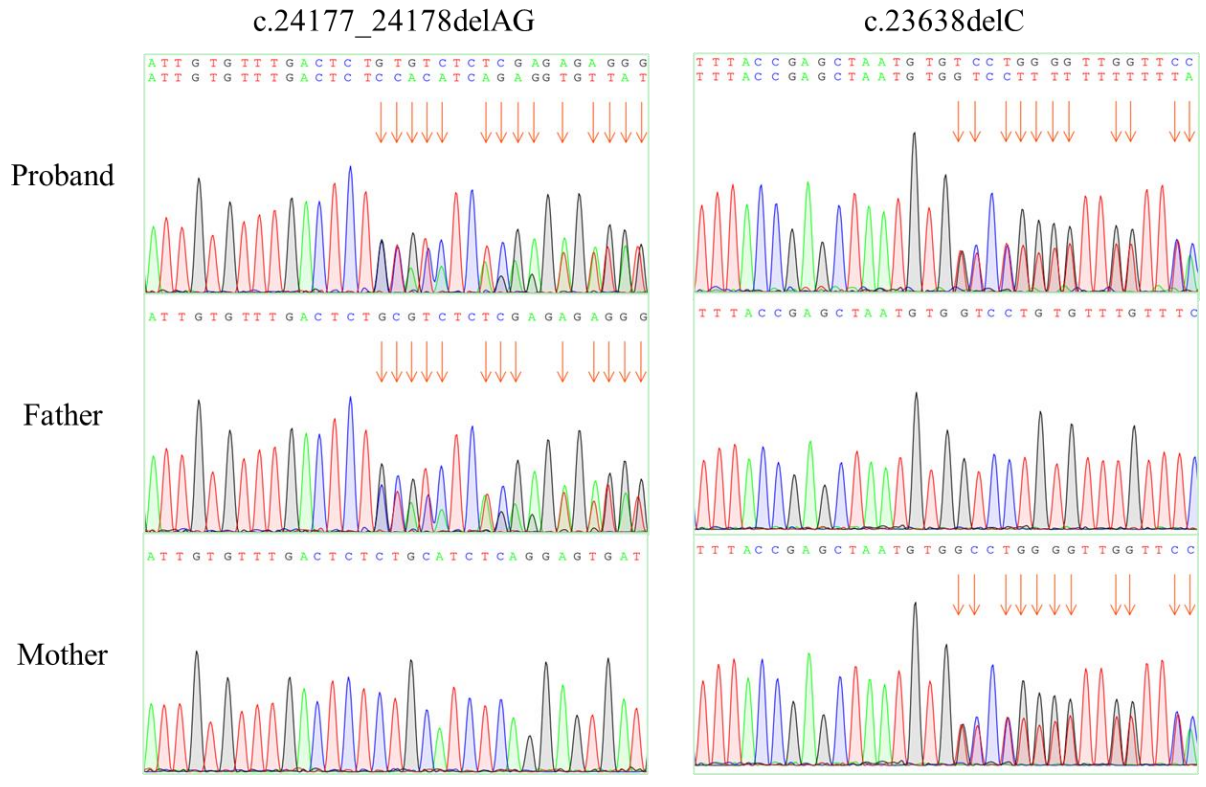

Figure S4 Sanger sequencing results for *NEB* gene variants in Patient 4 revealed that the c.23638delC was inherited from her mother, and the c.24177_24178delAG was inherited from her father.

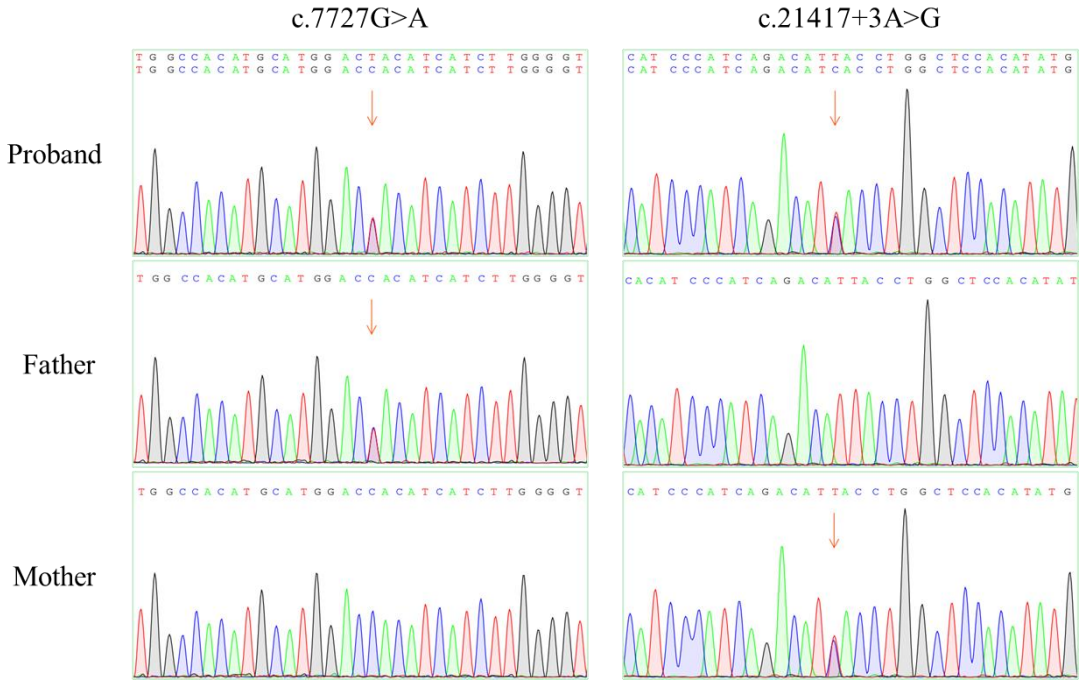

Figure S5 Sanger sequencing results of the *NEB* gene variants in Patient 5 revealed that the c.21417+3A>G variant was inherited from her mother, while the c.7727G>A variant was inherited from her father.

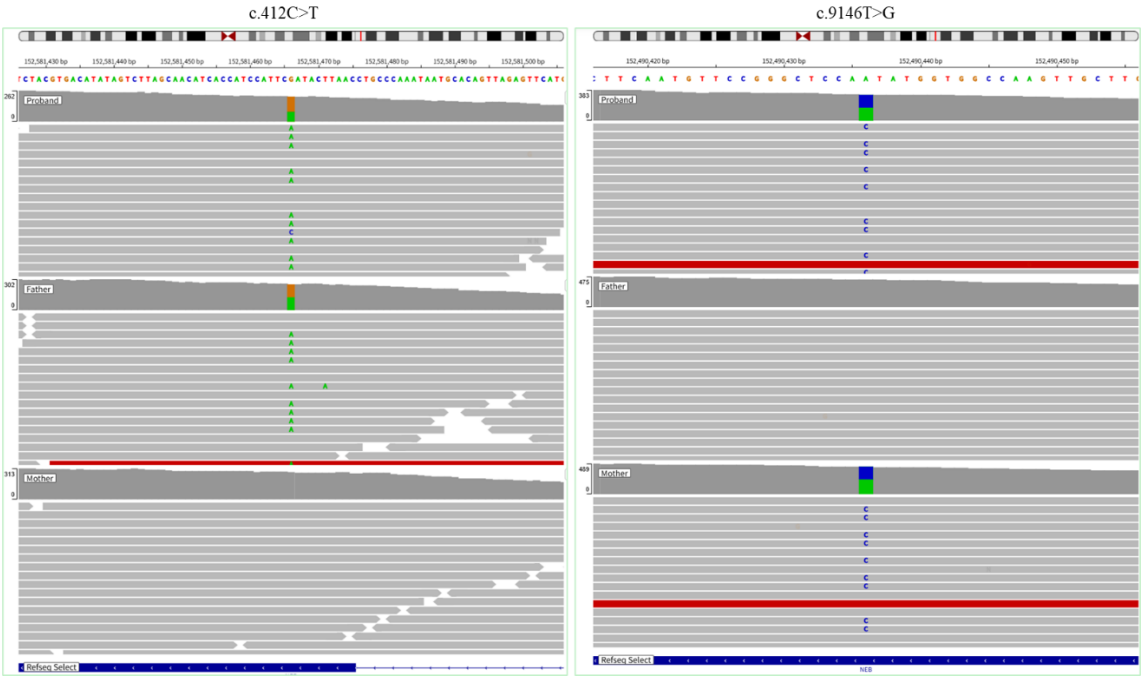

Figure S6 Next-generation sequencing results of the *NEB* gene variants in Patient 6 revealed that the c.9146T>G was inherited from her mother, and the c.412C>T was inherited from her father.

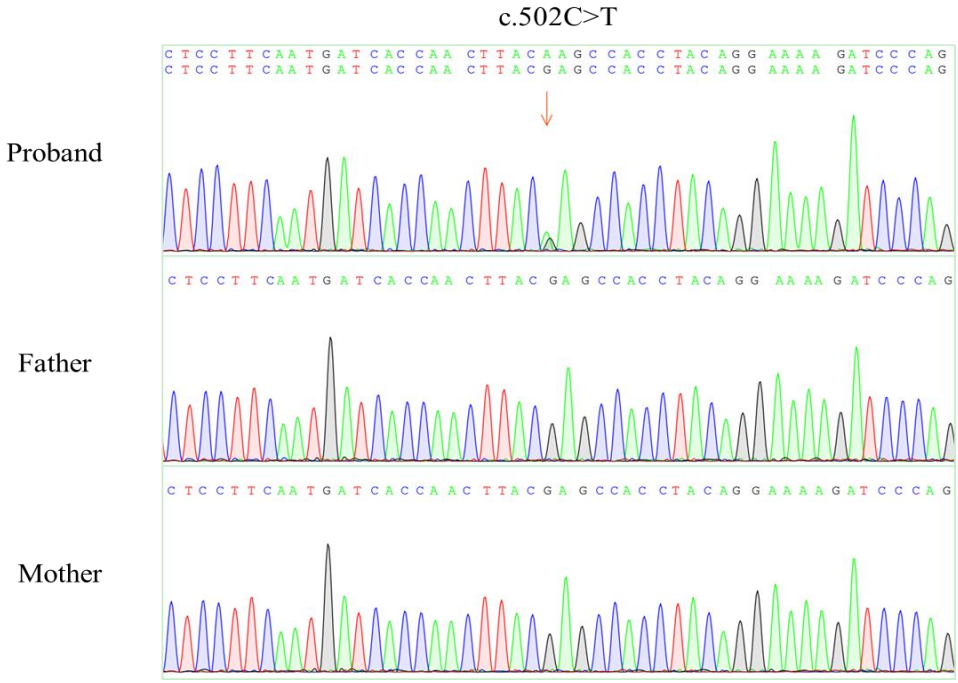

Figure S7 Sanger sequencing results of the *TPM3* gene variant in Patient 7. The c.502C>T was a *de novo* variant and both parents were wild-type.

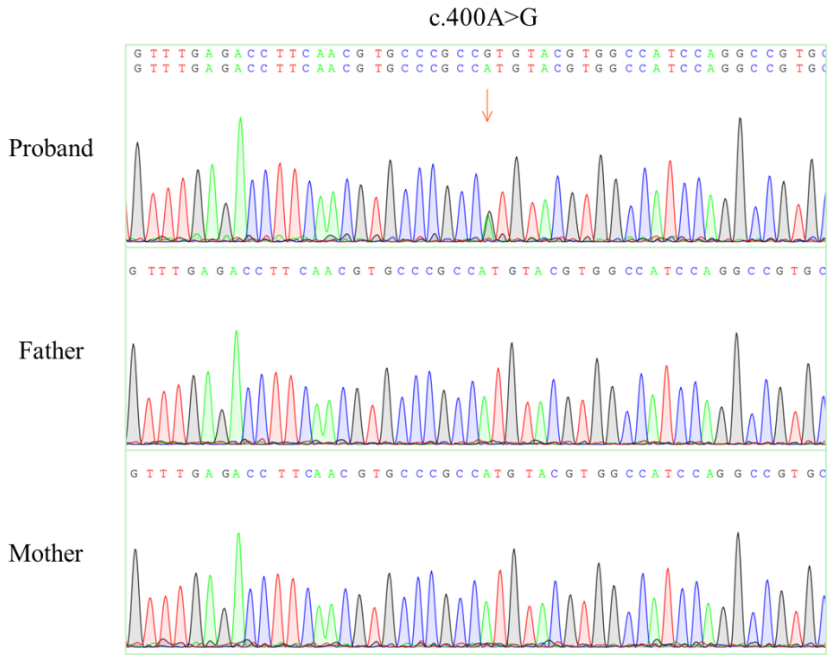

Figure S8 Sanger sequencing results of the *ACTA1* gene variant in Patient 8 indicated that the c.400A>G was a *de novo* variant, with both parents being of the wild - type.

c.21417+3A>G

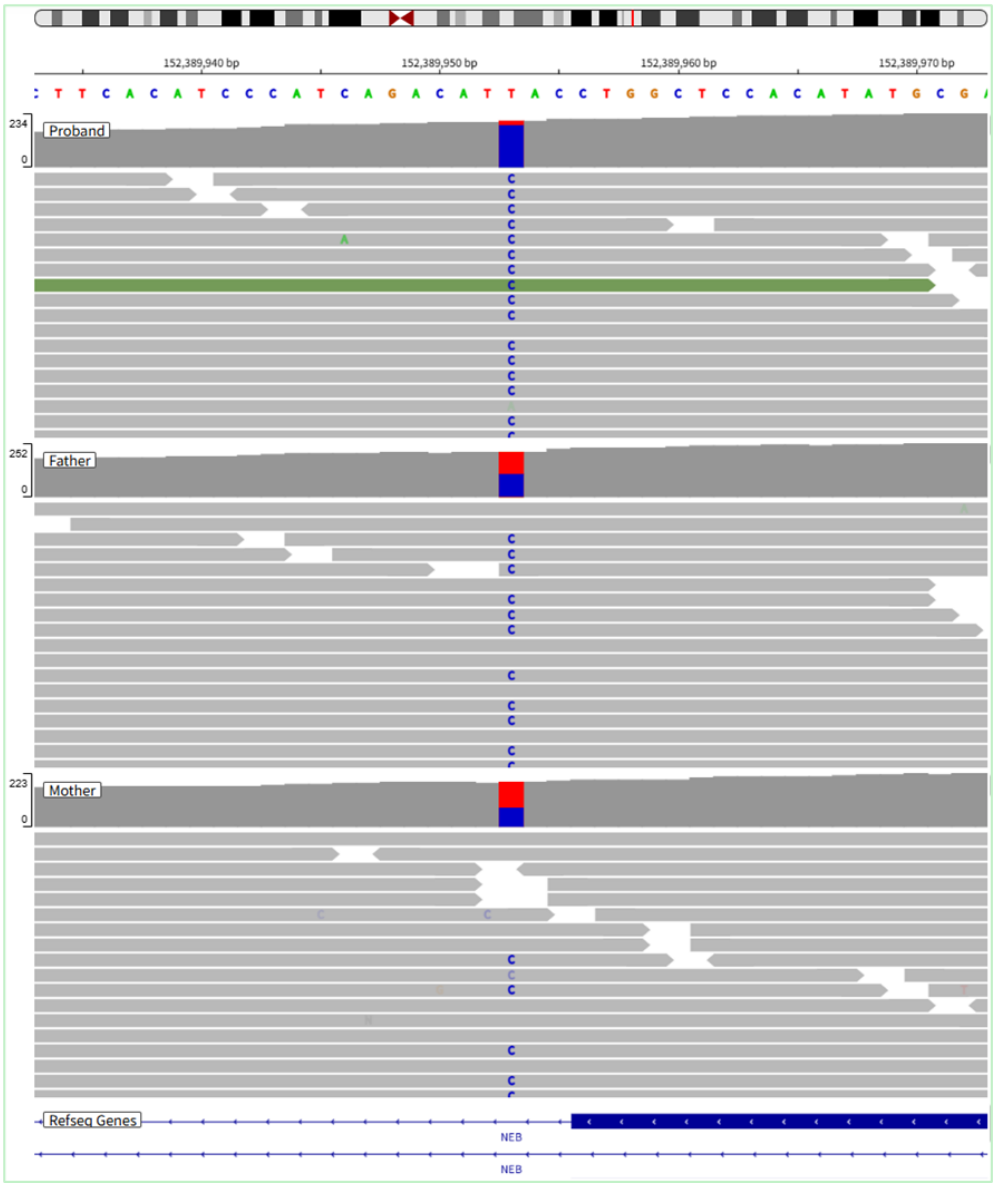

Figure S9 Next-generation sequencing results of the *NEB* gene variant in Patient 9 were visualized via the Integrative Genomics Viewer (IGV). The c.21417+3A>G was a homozygous variant, which was inherited from both parents.

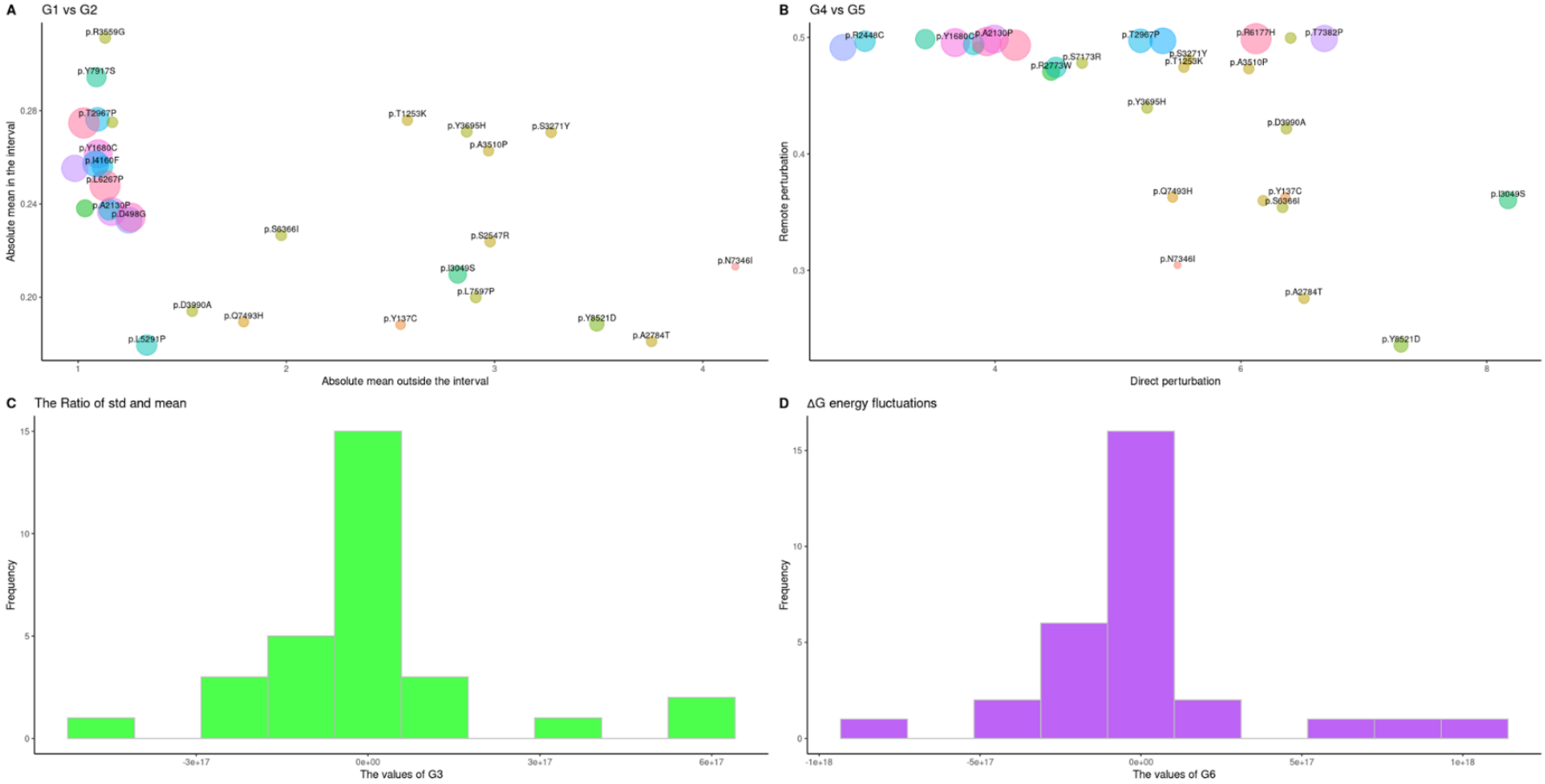

Figure S10 Thermodynamic Analysis Profiles of Nebulin Proteins with *NEB* Gene Missense Variants. A. indicated the average magnitude of abnormal fluctuations when there were significant deviations from the mean, as well as the average magnitude of energy changes within the normal range of fluctuations for each variant (G1 vs G2). B. denotes the amplitude of the most significant perturbation and the largest fluctuation for each variant(G4 vs G5). C.The relative degree of fluctuation(G3). D. The combined relative fluctuation (G3) is to be considered in conjunction with the differences in fluctuation both within and outside the range (the relative gap between G1 and G2).