

Case Report

The Homozygous c.612C>G Mutation in C1QBP is Associated with Late-Onset Progressive External Ophthalmoplegia

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Abstract

Biallelic pathogenic variants in C1QBP are an infrequent, yet increasingly recognised, cause of progressive external ophthalmoplegia (PEO). Although most published cases include cardiomyopathy, isolated late-onset PEO without cardiac disease remains exceptional. To characterise, for the first time, a Moldavian-Ukrainian pedigree in which homozygous c.612C>G (p.Phe204Leu) in C1QBP causes autosomal-recessive PEO in the absence of cardiomyopathy, thereby expanding the phenotypic spectrum of C1QBP-related disease. We performed a comprehensive clinical evaluation, needle electromyography, and targeted next-generation sequencing with segregation analysis in seven relatives, complemented by short-tandem-repeat profiling to assess parental relatedness. Three siblings (15–28 y) were homozygous for c.612C>G; four relatives were heterozygous and asymptomatic. Affected individuals shared bilateral ptosis, variable limb-girdle weakness, and gastrointestinal dysmotility, but routine echocardiography and electrocardiography were normal. EMG



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showed chronic myopathic potentials with reduced duration/amplitude in distal lower-limb muscles. In-silico prediction, extremely low allele frequency (0.005%), and segregation fulfilled ACMG class 5 (pathogenic). Our report represents one of the few families with C1QBP-related PEO without cardiomyopathy reported to date. These findings underline the importance of including C1QBP in diagnostic panels for apparently isolated PEO and illustrate the utility of family-based genome analysis for precision counselling and management.

Keywords

C1QBP; primary mitochondrial myopathy; progressive external ophthalmoplegia (PEO)

1. Introduction

The *C1QBP* gene, located on the short arm of 17p13.2, encodes the complement component 1Q subcomponent-binding protein (*C1QBP*, p32). It is highly conserved through evolution [1]. This widely expressed multifunctional protein is predominantly localized in the mitochondrial matrix [2]. *C1QBP* plays a crucial role in the oxidative phosphorylation of mitochondria (OXPHOS). Reduced OXPHOS and enhanced glycolysis correlate with mitochondria fragmentation and mitochondrial matrix expansion [3]. Its function is essential for maintaining standard mitochondrial structure and protecting mitochondria from fragmentation. Deficiency of C1QBP manifests as a mitochondrial disorder affecting multiple organs, including the heart, liver, skeletal muscle, eye, and nervous system. The identified disease-associated mutations in the *C1QBP* gene have an autosomal recessive type of inheritance. Biallelic *C1QBP* mutations were reported to cause mitochondrial cardiomyopathy and/or progressive external ophthalmoplegia (PEO), with variable age of onset and severity [4-9]. To date, only 13 patients with homozygous or compound heterozygous mutations have been documented, involving nine pathogenic variants of the *C1QBP* gene. Initial reports [4, 7, 8] highlighted associations with disruptions in the cardiovascular system, leading to early childhood mortality in half of the cases. Recent studies ([3, 7], our case report) now report *C1QBP* pathogenic variants in patients with no heart involvement. This data indicates that *C1QBP* mutations have to be considered in patients with isolated PEO and PEO-plus phenotype [9].

Advances in massively parallel sequencing have transformed the diagnostic approach to PEO: up to one-third of genetically unresolved cases harbour nuclear-encoded mitochondrial variants that escape detection by single-gene tests. Family-based genome interrogation not only accelerates diagnosis but also guides surveillance for systemic complications and informs reproductive choices.

Therefore, the present study set out to report the first detailed clinical-genetic characterisation worldwide of a family in which homozygous c.612C>G in *C1QBP* results in late-onset PEO without cardiomyopathy. Compared with previous single-patient descriptions, our pedigree — the largest sibship (n = 3) with C1QBP-related PEO studied so far — provides segregation evidence across three affected and four unaffected relatives, electromyographic quantitation of the myopathy, and a demonstration that unrelated parents can both carry this ultra-rare allele, underscoring the need for broad population screening.

Here, we describe a family of mixed Moldavian-Ukrainian ancestry residing in the Odesa and Lviv regions with late-onset PEO due to homozygous c.612C>G in *C1QBP*.

2. Materials and Methods

Seven family members (three affected, four unaffected) participated after written informed consent; the study adhered to the Declaration of Helsinki and required no further ethics-board approval.

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes of seven family members using the salting-out extraction method. DNA quantity and purity were measured by ultraviolet spectrophotometry (NanoDrop, Thermo Fisher Scientific) and fluorometric quantification (Qubit, Thermo Fisher Scientific).

Genotyping of the proband was carried out by next-generation sequencing (NGS) with the Nuclear Mitochondrial Disorders Panel on the Illumina MiSeq platform (paired-end reads 2×150 bp; mean depth $>100\times$) [10]. Reads were aligned to the human reference genome GRCh37 (hg19) using the Burrows–Wheeler Aligner with maximal exact matches (BWA-MEM) algorithm [11]. Single-nucleotide and indel variants were identified by the Genome Analysis Toolkit (GATK) HaplotypeCaller [12] and functionally annotated with ANNOVAR (ANNOtate VARIation) [13]. Variants were filtered by read depth $>20\times$, minor allele frequency $<1\%$ in the Genome Aggregation Database (gnomAD), and predicted deleteriousness. Pathogenicity was interpreted according to the 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines [14].

Candidate variants, including the homozygous c.612C>G substitution in the *C1QBP* gene, were confirmed by Sanger sequencing using a SeqStudio™ Genetic Analyzer (Applied Biosystems) and the BigDye™ Terminator v3.1 Cycle Sequencing Kit. Primers were designed with Primer3Plus [15]; their specificity was assessed by in-silico PCR and the Basic Local Alignment Search Tool (BLAST) [16]. Polymerase chain reaction (PCR) and sequencing amplicons were purified by ethanol precipitation, and chromatograms were reviewed in Chromas Lite.

Segregation analysis showed homozygosity for c.612C>G in all symptomatic individuals and heterozygosity in both parents and two unaffected siblings, consistent with autosomal-recessive inheritance.

Consanguinity assessment of the parental pair employed short tandem repeat (STR) profiling of 25 loci with the VeriFiler™ Plus Kit on the SeqStudio platform; allele comparison in VeriFiler™ software against European population frequencies (STRBase NIST archive) produced a likelihood ratio of 0.12, indicating little genetic relatedness.

Needle electromyography of distal and proximal limb muscles was performed (Medelec Synergy). Quantitative parameters (motor-unit potential duration, amplitude, polyphasicity) were exported for graphical analysis.

2.1 Ethics Statement

The study followed the Declaration of Helsinki. Written informed consent for genetic testing and publication was obtained from all participants or their legal guardians. Formal IRB approval was waived because this is a retrospective case report with no additional interventions.

3. Results

3.1 Clinical Findings (Proband and Siblings)

All three homozygous siblings exhibited a coherent PEO-plus phenotype that began in adolescence with bilateral ptosis, limb-girdle-predominant fatigability that curtailed running or stair-climbing, and episodic gastrointestinal dysmotility manifesting as morning nausea with or without vomiting. Ocular motility was variably restricted, yet none experienced diplopia. Resting serum creatine-phosphokinase remained within normal limits (≤ 162 U/L), and serial echocardiography and electrocardiography showed no evidence of cardiac involvement. Both parents and two heterozygous siblings were clinically unaffected. Individual highlights are summarised below.

The proband (II-3, female, 28 years) first noticed exercise-induced ptosis and limb fatigue at 14 years. During her third decade, the disorder progressed to dysphagia for solids, intermittent rhinolalia, exertional dyspnoea, morning sickness, and vomiting after maximal effort. A diagnosis of seronegative myasthenia gravis led to thymectomy at 20 years and treatment with pyridostigmine bromide 240 mg/day, neither of which improved symptoms. Neurological examination revealed symmetrical ptosis (palpebral fissure 3 mm; maximal up-gaze 6 mm), partial ophthalmoplegia without diplopia, hypophonia, nasalia, and mild lower-facial weakness. Serum CK was 111 U/L. Needle EMG of the tibialis anterior demonstrated a 30% reduction in mean motor-unit duration and a 35% reduction in amplitude, with marked polyphasicity.

The elder brother (II-4, male, 22 years) developed bilateral ptosis at 16 years, accompanied by chronic exertional myalgia, calf cramps, and post-exercise tachycardia. EMG reproduced the proband's pattern, showing a 45% reduction in tibialis anterior amplitude and relative increases in vastus lateralis duration (+12%) and amplitude (+79%). Serum lactate was mildly elevated at 4.36 mmol/L.

The younger sister (II-5, female, 15 years) currently attends school with only subtle early-morning fatigue and nausea; neurological examination is regular, and neither ptosis nor ophthalmoparesis is present, illustrating intra-familial variability despite an identical genotype.

Electromyography across the sibship consistently showed reduced mean motor-unit duration ($\approx 30\%$) and amplitude ($\approx 35\text{--}45\%$) in the anterior tibial muscles, combined with increased polyphasicity—classical indicators of chronic myopathic remodelling in mitochondrial disease that correlate with the distal-leg weakness typical of PEO (Figure 1).

left, Tibialis anterior, Peroneus, L4 L5 s1**Motor unit potential duration**

Parameter	Value
Minimum duration, ms	4,76
Maximum duration, ms	8,74
Average duration, ms	7,62
Standard duration, ms	10,9
Duration deviation, %	-30,1
Number of motor unit potentials	8

Motor unit potential amplitude

Parameter	Value
Minimum amplitude, μV	308
Maximum amplitude, μV	645
Average amplitude, μV	484
Standard amplitude, μV	750
Amplitude deviation, %	-35,4
Polyphasicity, %	25,0

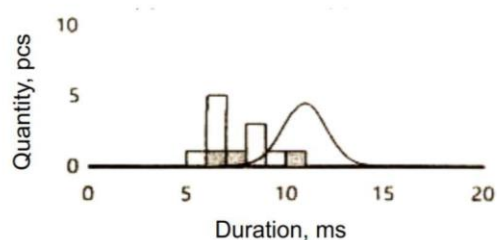
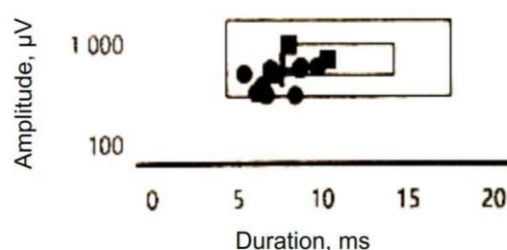
Motor unit potential duration**Duration-amplitude of motor unit potentials**

Figure 1 Quantitative electromyography of the left tibialis anterior muscle. The figure includes raw motor unit potentials (top), duration and amplitude tables (left and center), and graphical distribution of motor unit duration and duration-amplitude correlation (right). Data illustrate significantly reduced mean duration and amplitude relative to normal values.

It was recommended that all affected individuals take coenzyme Q10 at a dose of up to 1200 mg per day continuously [17].

3.2 Genetic Analysis

Next-generation sequencing (NGS) using the Comprehensive Neuromuscular Disorders Panel identified a homozygous c.612C>G (p.Phe204Leu) substitution in the *C1QBP* gene (NM_001212.4). The variant is exceedingly rare (allele frequency 0.005% in gnomAD) and is classified as pathogenic (class 5) according to ACMG/AMP 2015 guidelines. However, conflicting interpretations are reported in ClinVar. In-silico prediction tools support its pathogenicity: SIFT = 0.01 (deleterious), PolyPhen-2 = 0.999 (probably damaging), and HOPE predicted significant structural destabilization.

Segregation analysis by Sanger sequencing revealed homozygosity for the c.612C>G variant in the proband and two affected siblings, and heterozygosity in both asymptomatic parents and two unaffected siblings (Figure 2). Sequencing chromatograms illustrating the c.612C>G variant are shown in Figure 2.

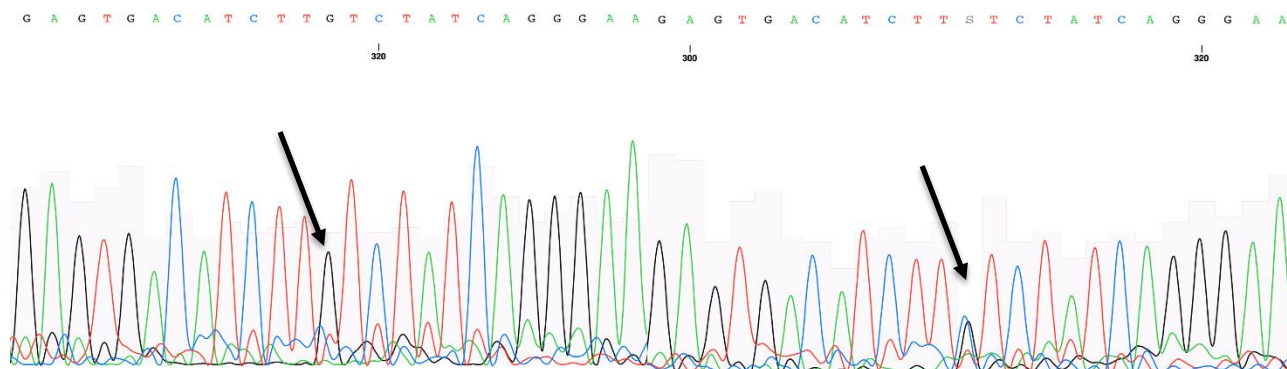


Figure 2 Sanger sequencing chromatograms of the c.612C>G variant in the C1QBP gene.

To assess potential consanguinity, short tandem repeat (STR) profiling of 25 loci was performed for both parents using the VeriFiler™ Plus Kit. Comparative allelic analysis across all loci revealed a low degree of overlap (Table 1). VeriFiler software calculated a likelihood ratio (LR) of 0.12, indicating no significant genetic relatedness between the individuals. STR allele frequencies were referenced from the European population dataset available at the STRBase NIST Archive (<https://strbase-archive.nist.gov>).

Table 1 STR-loci profiling of the proband's parents using the VeriFiler™ Plus Kit.

Marker	33597		33598		Coincidence
	Allele 1	Allele 2	Allele 1	Allele 2	
D3S1358	14	15	15	16	yes
vWA	14	16	16	17	yes
D16S539	12	13	12	12	yes
CSF1PO	12	12	11	11	no
TPOX	8	9	8	11	yes
Yindel			2		no
AMEL	X	X	X	Y	yes
D8S1179	13	16	12	12	no
D21S11	29	32.2	30	30.2	no
D18S51	15	15	14	15	yes
Penta E	7	15	7	16	yes
D2S441	10	11	11	12	yes
D19S433	13	15	14	15.2	no
TH01	9	9.3	6	9.3	yes
FGA	20	23	22	25	no
D22S1045	11	15	16	16	no
D5S818	9	12	12	14	yes
D13S317	12	12	12	12	yes
D7S820	8	10	9	12	no
D6S1043	12	17	12	20	yes
D10S1248	13	13	14	16	no
D1S1656	15.3	17	14	17.3	no

D12S391	22	22	19	23	no
D2S1338	19	23	24	25	no
Penta D	11	13	9	13	yes

Two affected siblings, aged 22 and 15, demonstrated similar clinical symptoms. The 22-year-old male (homozygous) complains of constant drooping of the eyelids, muscle weakness (he cannot run or walk quickly), morning sickness, vomiting, tachycardia after maximum physical exertion for him, sometimes with muscle fatigue, and calf muscle cramps occur. Muscle weakness, nausea, and vomiting have been observed since childhood, and ptosis has developed since the age of 16. Objectively, there was bilateral partial ptosis, partial ophthalmoparesis without diplopia, but there were no bulbar disorders and facial paresis.

The results of the electroneuromyographic examination were identical: there was no decrement during rhythmic stimulation, in the left musculus tibialis anterior motor unit potentials were reduced in amplitude to 410 μ V (average value) at the normal of 750 μ V, which corresponds to a decrease of 45.4%, in the musculus vastus lateralis motor unit potentials were increased in duration to 11.4 ms (average indicator), at the age normal for this patient of 10.2 ms, which corresponds to an increase of 11.5%, and by amplitude to 1339 μ V (average indicator), at a normal of 750 μ V, which corresponds to a rise of 78.5%. The levels of key laboratory indicators are indicated in Table 2.

Table 2 Levels of key laboratory indicators of a 22-year-old male patient.

Indicator	Results	Unit	Normal	Unit
Coenzyme Q10	905	μ g/l	750.0-1000.0	μ g/l
Pyruvate (pyruvic acid)	103.8	mmol/l	24.0-95.0	mmol/l
Lactate	4360	mmol/l	525.0-1520.0	mmol/l
Lactate-pyruvate ratio	42		12.1-24.2	
Lactate in the urine	8.9	mg/g Krea	2.4-8.7	mg/g Krea
Pyruvate in urine	8.3	mg/g Krea	3.2-6.4	mg/g Krea
Lactate-pyruvate ratio in urine	1.07		0.5-1.4	
Creatine phosphokinase	162	U/L	$\leq$ 171.0	U/L

Ophthalmological examination revealed complex hyperopic astigmatism on the correct eye and mixed astigmatism on the left eye. Electromyography results were similar. Symptomatic treatment was carried out, and motor activity was limited, which led to increased muscle weakness and disability.

The 15-year-old female (homozygous) presented with mild generalized weakness and morning nausea. She attends school and participates in daily activities, although she avoids intensive physical exercise. No facial or ocular involvement has been noted to date.

Parents and two heterozygous siblings are clinically asymptomatic; the mother suffers from gastritis. A summary of genotype, clinical status, and laboratory findings of all family members is provided in Table 3.

Table 3 Genotype, clinical features, and laboratory findings of family members.

ID	Age/Sex	Genotype	Clinical status	Ptosis	Muscle weakness	GI symptoms	CPK (U/L)	Lactate (μmol/L)	EMG Finding
Proband	28F	homozygous	Symptomatic	Yes	Yes	Yes	111	-	Myopathic
Sibling 1	22M	homozygous	Symptomatic	Yes	Yes	Yes	162	4360	Myopathic
Sibling 2	15F	homozygous	Mild symptoms	No	Mild	Nausea	-	-	N/A
Sibling 3	27F	heterozygous	Asymptomatic	No	No	No	-	-	N/A
Sibling 4	35M	heterozygous	Asymptomatic	No	No	No	-	-	N/A
Mother	55F	heterozygous	Asymptomatic	No	No	Gastritis	-	-	N/A
Father	63M	heterozygous	Asymptomatic	No	No	No	-	-	N/A

CPK – creatine phosphokinase; EMG – electromyography; GI – gastrointestinal; N/A – not available; “↑” – increased; “-” – not tested.

The family tree is shown in Figure 3.

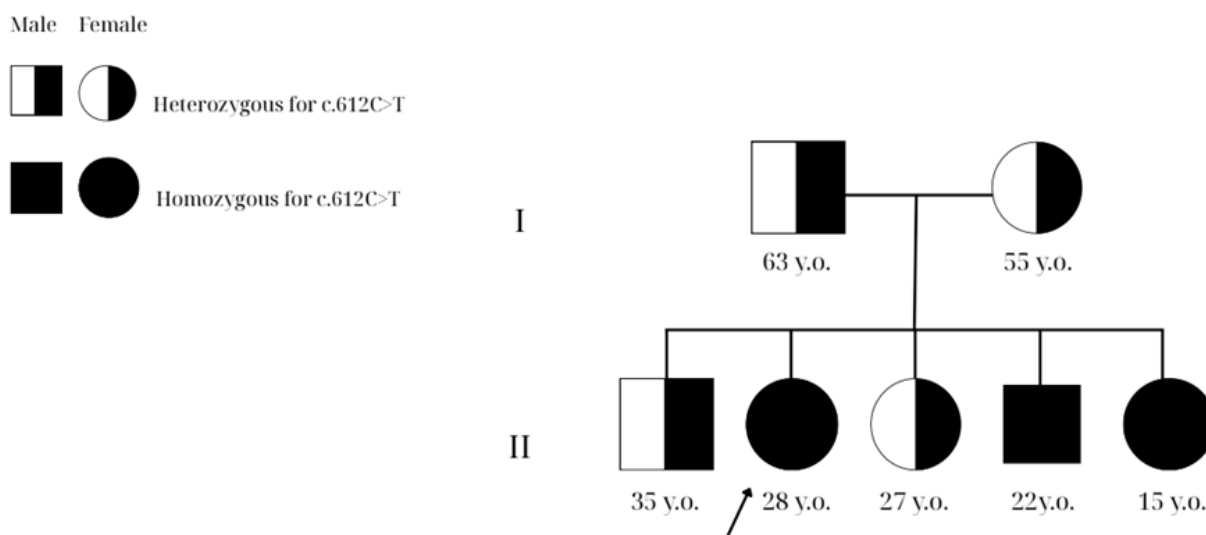


Figure 3 The family tree shows affected individuals homozygous for the c.612C>G mutation (entirely black), heterozygous carriers (half-filled), and the proband (arrow). Squares represent males; circles represent females.

Variant interpretation. According to the 2015 ACMG/AMP guidelines [14], the homozygous c.612C>G (p.Phe204Leu) variant meets the following evidence codes: PS1 (Strong) – identical amino-acid change to a previously established pathogenic variant via a different nucleotide substitution; PM2 (Moderate) – absent or extremely rare in population databases (gnomAD allele frequency = 0.00005, i.e. 0.005%); PM3 (Moderate) – observed in the homozygous state in three affected siblings with an autosomal-recessive phenotype; PP1 (Supporting) – co-segregation with disease across more than three informative meioses; PP5 (Supporting) – reported as pathogenic in multiple curated clinical databases (e.g. ClinVar, LOVD). The combination of 1 Strong + 2 Moderate + 2 Supporting criteria fulfils the threshold for a pathogenic (class 5) classification.

4. Discussion

Notably, a single known patient with the homozygous c.612C>G (p.Phe204Leu) variant [5], as seen in our cohort, exhibited a late onset of symptoms (including bilateral ptosis, ophthalmoparesis, muscle weakness, and rhinolalia) without reported cardiac involvement.

Before molecular confirmation, differential diagnoses included seronegative myasthenia gravis, oculopharyngodistal myopathy, and mitochondrial DNA (mtDNA) deletion syndromes. Negative AChR/MuSK antibodies, lack of fatigability on repetitive-nerve stimulation, absence of pearl-string myotonic discharges, and normal ptosis response to neostigmine test argued against myasthenia. mtDNA long-range PCR and a 74-gene “PEO/Multiple-mtDNA-deletions” NGS panel revealed no rearrangements or alternative nuclear defects, effectively ruling out POLG, TWNK, DNA2, and SPG7-related phenocopies.

This suggests a potentially milder and benign course of the disease when the homozygous c.612C>G mutation is present. Guo L et al. [9] also reported a patient with late-onset PEO who exhibited no overt cardiac symptoms but was found to have a homozygous deletion variant

c.611_613del in *C1QBP*, resulting in the loss of Phe204. This could be attributed to the altered or deleted amino acid p.Phe204 being located in the coiled-coil region rather than in critical structural domains. In contrast, variants such as p.Cys186Ser, p.Gly247Trp, p.Val248Ala, p.Thr40Asnfs*45, p.Leu275Phe, and p.Leu275Pro, which are associated with early disease onset, particularly cardiomyopathy, are found in critical structural domains, such as the β -strand, hydrogen-bonded turn, and α C-helix [6]. The location of the variant could be essential to explain or predict the different clinical manifestations.

Although no genotype-directed therapy exists, mitochondrial cocktail supplementation remains a pragmatic approach. We initiated high-dose coenzyme Q10 (up to 1200 mg/day) based on favourable outcomes reported in open-label and randomized studies and its role in electron-transport-chain shuttling, with evidence of symptomatic benefit in select mitochondrial myopathies [17]. Prospective follow-up will clarify whether early implementation attenuates progression, particularly in the minimally affected 15-year-old carrier.

5. Conclusion

In conclusion, our data broaden the genotype-phenotype correlation of *C1QBP* by demonstrating that homozygous c.612C>G can manifest as pure, late-onset PEO without cardiac involvement. The findings advocate for the systematic inclusion of *C1QBP* in NGS panels for all PEO presentations, even when cardiomyopathy is absent, and illustrate how family-centered genome sequencing refines prognosis, guides targeted surveillance, and informs carrier testing in extended relatives.

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Author Contributions

Ivanna Shymanska: Developed the Sanger sequencing design, wrote the "materials and methods" section. Sofiia Levandivska: collected and analyzed literature data, wrote the text of the article. Marharyta Marchuk: Patient consultation, collection and analysis of data from larotatory studies, writing the text of the article. Anastasiya Honchar: Patient consultation, collection and analysis of data from larotatory studies, writing the text of the article. Volodymyr Kravets determined the degree of kinship between parents to exclude consanguineous marriage. Halyna Makukh was responsible for project development.

Competing Interests

The authors declare no conflicts of interest.

References

1. Guo N, Weremowicz S, Lynch N, Lim BL. Assignment (a) of *C1QBP* encoding the C1q globular domain binding protein (gC1q-R) to human chromosome 17 band p13. 3 by in situ hybridization. *Cytogenet Genome Res.* 1997; 77: 283-284.

2. Dedio J, Jahnen-Dechent W, Bachmann M, Muller-Esterl W. The multiligand-binding protein gC1qR, putative C1q receptor, is a mitochondrial protein. *J Immunol.* 1998; 160: 3534-3542.
3. Alirol E, Martinou JC. Mitochondria and cancer: Is there a morphological connection? *Oncogene.* 2006; 25: 4706-4716.
4. Feichtinger RG, Oláhová M, Kishita Y, Garone C, Kremer LS, Yagi M, et al. Biallelic C1QBP mutations cause severe neonatal-, childhood-, or later-onset cardiomyopathy associated with combined respiratory-chain deficiencies. *Am J Hum Genet.* 2017; 101: 525-538.
5. Marchet S, Legati A, Nasca A, Di Meo I, Spagnolo M, Zanetti N, et al. Homozygous mutations in C1QBP as cause of progressive external ophthalmoplegia (PEO) and mitochondrial myopathy with multiple mtDNA deletions. *Hum Mutat.* 2020; 41: 1745-1750.
6. Wang J, Li H, Sun M, Yang Y, Yang Q, Liu B, et al. Early onset of combined oxidative phosphorylation deficiency in two chinese brothers caused by a homozygous (Leu275Phe) mutation in the C1QBP gene. *Front Pediatr.* 2020; 8: 583047.
7. Alstrup M, Vogel I, Sandager P, Blechingberg J, Becher N, Østergaard E. A novel homozygous variant in C1QBP causes severe IUGR, edema, and cardiomyopathy in two fetuses. *JIMD Rep.* 2021; 59: 20-25.
8. Webster G, Reynolds M, Arva NC, Dellefave-Castillo LM, McElligott HS, Kofman A, et al. Mitochondrial cardiomyopathy and ventricular arrhythmias associated with biallelic variants in C1QBP. *Am J Med Genet A.* 2021; 185: 2496-2501.
9. Guo L, Govindaraj P, Kievit M, de Coo IF, Gerards M, Hellebrekers DM, et al. Whole exome sequencing reveals a homozygous C1QBP deletion as the cause of progressive external ophthalmoplegia and multiple mtDNA deletions. *Neuromuscul Disord.* 2021; 31: 859-864.
10. Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature.* 2008; 456: 53-59.
11. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv.* 2013. doi: 10.48550/arXiv.1303.3997.
12. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet.* 2011; 43: 491-498.
13. Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010; 38: e164.
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015; 17: 405-423.
15. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. Primer3—New capabilities and interfaces. *Nucleic Acids Res.* 2012; 40: e115.
16. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol.* 1990; 215: 403-410.
17. Glover EI, Martin J, Maher A, Thornhill RE, Moran GR, Tarnopolsky MA. A randomized trial of coenzyme Q10 in mitochondrial disorders. *Muscle Nerve.* 2010; 42: 739-748.