Case Report

Whole exome sequencing reveals a homozygous C1QBP deletion as the cause of progressive external ophthalmoplegia and multiple mtDNA deletions

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Abstract

Whole exome sequencing (WES), analyzed with GENESIS and WeGET, revealed a homozygous deletion in the C1QBP gene in a patient with progressive external ophthalmoplegia (PEO) and multiple mtDNA deletions. The gene encodes the mitochondria-located complementary 1 Q subcomponent-binding protein, involved in mitochondrial homeostasis. Biallelic mutations in C1QBP cause mitochondrial cardiomyopathy and/or PEO with variable age of onset. Our patient showed only late-onset PEO-plus syndrome without overt cardiac involvement. Available data suggest that early-onset cardiomyopathy variants localize in important structural domains and PEO-plus variants in the coiled-coil region. Our patient demonstrates that C1QBP mutations should be considered in individuals with PEO with or without cardiomyopathy.

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1. Introduction

Progressive external ophthalmoplegia (PEO; OMIM PS157640) is a group of disorders characterized by progressive ptosis and decreased ocular motility, occurring in isolation (PEO-only) or accompanied with other mitochondria-related symptoms (PEO-plus) [1]. PEO exhibits considerable genetic heterogeneity. Approximately half of the PEO patients are sporadic, presenting with a single large mtDNA deletion [1,2]. The other half is either maternally inherited or has autosomal dominant (AD), or autosomal recessive (AR) inheritance, associated with multiple mtDNA deletions. Maternally inherited PEO, either isolated or part of a multi-systemic phenotype, is caused by point mutations in mtDNA. AD-PEO is caused by mutations in at least seven nuclear genes, including POLG, POLG2, SLC25A4, TWNK, RRM2B, DNA2, and OPA1. In contrast, AR-PEO is less common and can result from mutations in nuclear genes such
as TYP, DGUOK, TK2 and RNASEH1. All of the causative nuclear genes linked to PEO encode proteins required for mtDNA replication and maintenance [3].

Whole exome sequencing (WES) has become the standard for identifying causative variants in genetically heterogeneous diseases, like PEO. However, bioinformatics analysis to discover causative variants remains challenging. Therefore, two bioinformatic tools, GENESIS and WeGET, were applied. GENESIS is a next-generation sequencing data-sharing platform integrated with various variants filtering tools [4]. Until now, over 160 studies have used or cited GENESIS, enabling the discovery of 72 novel pathogenic genes and/or expanding their genotype/phenotype relationship. WeGET is a computational tool to identify novel candidate genes based on co-expression with a query gene set of interest [5].

We combined GENESIS and WeGET for WES data analysis in a patient with PEO-plus and multiple mtDNA deletions. We discovered a novel homozygous deletion in the C1QBP gene, recently reported as a cause of PEO and/or cardiomyopathy [6,7].

2. Case report

A female patient (aged 33, pedigree Fig. 1A) reported mild intermittent headache for two years, drooping of eyelids for 1.5 years and swallowing difficulty for six months at first visit. At follow up after three and a half years she reported improvement in headache but she had nasal speech and there was a mild progressive proximal upper and lower muscle weakness against resistance (MRC scale:4/5). Further information on the death of the patient’s niece (pedigree Fig. 1A) was unknown, as the rest of the family members declined further testing.

Apart from a mild high arch palate, her general medical examination was normal. Neurological examination revealed normal visual acuity and fundi. She had bilateral symmetrical mild ptosis and ophthalmoparesis. Facial, neck flexor, finger extenders and hip flexor showed muscle weakness, whereas bulbar and other limb muscles showed normal strength. The sensory system was normal. All tendon reflexes were 1+ except for ankle jerks which were normal. The plantar response was flexor. There was no appreciable fatigue on repeated efforts.

Laboratory tests showed elevated creatine kinase (1032 U/L, normal<145 U/L), FGF21 (3351.26 pg/ml, normal<331 pg/ml) and GDF15 (5891.39 pg/ml, normal <1014 pg/ml), and low B12 level (101.8 pg/ml, normal>180 pg/ml) in serum. Motor and sensory conduction study and repetitive nerve stimulation test from nasalis, orbicularis oculi, and abductor digitii minimi muscles were normal. Electro/echocardiographic studies were normal. Brainstem auditory and somatosensory evoked potentials were normal. Visual evoked potential (VEP) study was performed on both sides and VEP results showed a mild delay in P100 latency on the right side (P100: rt. 113.3 ms, lt. 105.9 ms, normal<108 ms). Electroretinography was not done. MRI of the brain showed early mineralization of globi pallidi and dentate nuclei with normal MR spectroscopy. Biceps muscle biopsy revealed nearly 25% ragged-red (Fig. 1B, modified Gomori trichrome, MGT) and ragged-blue fibers (Fig. 1B, succinate dehydrogenase, SDH) and 50% cytochrome c oxidase (COX)-negative fibers (Fig. 1B, COX-SDH). Electron microscopy (EM) showed aggregates of a large number of mitochondria of varying size, altered cristae, parking lot inclusions and unusual triangular crystalline inclusions (Fig. 1B, EM). Complex IV deficiency (entire complex IV) was detected in muscle biopsies. Respiratory chain complex assays were done using spectrophotometric analysis: complex I-0.12 (40.98%), II-0.26 (61.45%), III-0.34 (75.26%), IV-0.16 (15.66%). DNA studies revealed multiple mtDNA deletions in muscle (Fig. 1C).

The most common genetic cause of PEO/PEO plus patients are single mtDNA deletions, mtDNA point mutations and multiple deletions with POLG1 mutations. For cost/efficiency reasons, we started with this targeted analysis, followed by whole exome sequencing in case of a negative result. Exome sequencing was not adopted as the first line because of the cost involved and lack of availability at the time of evaluation. Pathogenic variants in mtDNA and POLG were excluded by Sanger sequencing. A clinical exome sequencing on 6883 genes was performed and ruled out the known common AD cause of PEO with multiple mtDNA deletions. Informed consent was obtained from the patient according to the Declaration of Helsinki.

Whole exome sequencing (WES) was performed using Illumina HiSeq2000 sequencer as described before [8]. Two GENESIS filter sets were used to analyze the WES data. The first ‘Inheritance Filter’ was predefined as ‘autosomal recessive, strict’ mode, including data quality, variant type and frequency, evolutionary conservation and recessive inheritance (Supplementary Table 1). The second ‘Mitochondrial Filter’ selected nuclear genes associated with mitochondrial or mitochondrial diseases from MSeqDR [9] and MitoCarta [10], prior to applying the predefined ‘autosomal recessive, strict’ filters.

Two gene lists were compiled as query genes for WeGET analysis. A custom-made list included reported PEO, mtDNA maintenance, mitochondrial myopathy associated genes [1,3,11] and MitoCarta [10]. A precomputed gene list using GO-terms ‘Mitochondrial organization’; KEGG pathway ‘DNA replication’; and REACTOME pathways ‘Mitochondrial biogenesis’ was directly accessed from WeGET database. Each gene list was cross-validated in WeGET with the AUC ≥ 0.75, and genes ranking outside the 500 highest co-expressed position were removed. The trimmed gene lists were loaded into the GENESIS with a less stringent set of filters (denoted as ‘WeGET Filter’, Supplementary Table 2).

Candidate variants after GENESIS and WeGET analysis were manually checked for (A) Minor allele frequency (MAF) in public databases including gnomAD (http://gnomad.broadinstitute.org/), 1000 Genome (https://www.internationalgenome.org/), Exome Variant Server (https://evs.gs.washington.edu/EVS/) and
Fig. 1. WES data analysis and genetic findings in the patient. (A) Pedigree of the patient family. The patient was depicted by the filled black symbol. (B) Transversely cut skeletal muscle tissue shows ragged red fibre by modified Gomori trichrome (MGT) staining (labeled as *1, ×200), ragged blue fibre by succinate dehydrogenase (SDH) staining (labeled as *2, ×200), cytochrome c oxidase (COX) negative fibre by COX-SDH double staining (COX-SDH) (labeled as *3, ×200) and electron microscopy (EM) shows abnormal mitochondria in size, altered cristae and parking lot inclusions indicated by white arrows. (C) Long-range PCR of mtDNA showing multiple deletions in the patient (Pt) muscle biopsies, which are absent in the control (Ct). (D) Electropherogram of the C1QBP regions containing the homozygous NM_001212.4:c.611_613del deletion in the patient. (E) Amino acid conservation study. The amino acid F204 (in bold) is conserved down to C. elegans, which is absent in the patient due to the NM_001212.3:c.611_613del variant. (F) Schematic view of C1QBP gene and all pathogenic variants identified to date. The variant in bold indicates the position of the amino acid changes present in the patient from this study.
Table 1
Predicted consequence of C1QBP variants.

| Item                                      | This patient-Allele 1+2 |
|-------------------------------------------|-------------------------|
| Mutation, cDNA (NM_001212.3)             | c.611_613delTCT         |
| Protein (NP_001203.1)                     | p.Phe204del             |
| Mutation, genomic (hg19)                  | chr17:5336699_5336701delAGA |
| Exon position of the mutation             | 5                      |
| gnomAD frequency                          | 0.000003976 (1:251480)  |
| 1000 genome frequency                     | Not available           |
| EVS frequency                             | Not available           |
| GENESIS                                   | 1 homozygous allele     |
| SIFT prediction                           | damaging                |
| SIFT confidence score                     | 0.894                   |
| Provean prediction                        | Deleterious             |
| Provean score (cutoff<-2.50)              | -12.61                  |
| CADD Phred score (cutoff>20)              | 22.70000076             |

GENESIS (https://www.tgperfoundation.org/g-e-n-e-s-i-s); (B) Predicted pathogenicity by CADD Phred (https://cadd.gs.washington.edu/), SIFT (https://sift.bii.a-star.edu.sg/), Provean (http://provean.jcvi.org-genome_submit_2.php?species=human); (C) Known pathogenicity in ClinVar and Leiden Open Variation Database (LOVD).

Based on the pedigree and consanguinity of the healthy parents, autosomal recessive inheritance and homozygosity of pathogenic variants seemed the most likely cause for the PEO in the patient (Fig. 1A). The ‘Inheritance Filter’, ‘Mitochondrial Filter’ and ‘WeGET Filter’ of WES data resulted in a total of 11 homoyzogous variants (Supplementary Table 3). Subsequent manual checking on frequency, allele count and predicted pathogenicity reduced the candidate variants to a homozygous deletion variant, NM_001212.3(C1QBP): c.611_613del. Sanger sequencing confirmed this homozygous deletion variant in patient (Fig. 1D). Segregation analysis was not available due to lack of materials from parents. Based on the following criteria of the American College of Medical Genetics criteria [12], our variant is classified as likely pathogenic:

- Strong evidence of pathogenicity (PS1): The variant p.Phe204del affects the same amino acid change as the functionally validated pathogenic variant p.Phe204Leu, which has been reported in 2 other patients (Feichtinger et al. [6] and Marchet et al. [7]).
- Moderate evidence of pathogenicity (PM2): This deletion was not present in 1000 Genomes, ClinVar or LOVD and only once as heterozygote in gnomAD (MAF=0.000004).
- Moderate evidence of pathogenicity (PM4): The in-frame deletion in a non-repeat region is predicted to cause a length change of C1QBP.
- Supporting evidence of pathogenicity (PP3): The loss of F204 affects a highly conserved amino acid and is predicted to be pathogenic according to SIFT, Provean and CADD (Table 1).

As the patient is homozygous for the deletion, it is highly likely that both unaffected parents are carrier of the deletion, which is in line with autosomal inheritance. This is also considered strong evidence for pathogenicity. We have reached out to the parents and remainder of the family, but in spite of our best efforts, the family members declined further testing, so we were unable to confirm the segregation of the variant in the family and get patient fibroblasts for functional studies. As complementation studies have been performed by others (Feichtinger et al. [6]), we consider this as supportive evidence as well.

3. Discussion

We combined GENESIS and WeGET to analyze WES data in an Indian patient with PEO and multiple mtDNA deletions for homozygous, autosomal recessive variants. After applying three different filtering strategies and manual confirmation, rare and evolutionary conserved variants were prioritized, leading to the discovery of a homozygous, likely pathogenic in-frame deletion in the C1QBP gene.

C1QBP encodes the complement component 1Q binding protein (C1QBP), also known as p32. C1QBP is a ubiquitous protein mainly localized in the mitochondrial matrix and involved in mitochondrial ribosome biogenesis [13]. Knockdown of C1QBP in HeLa cells caused fragmentation of the mitochondrial network, whereas overexpression of C1QBP showed a more fibrillar network [14]. Reduced levels of C1QBP in cardiac cells, neurons and dendritic cells resulted in both defective mitochondrial function and disturbed maturation, differentiation or signaling [15-17].

Biallelic C1QBP mutations were reported to cause mitochondrial cardiomyopathy and (or) PEO, with variable age of onset and severity (Table 2) [6,7,18]. So far, six pathogenic C1QBP variants have been reported from eight patients (Fig. 1C). C1QBP mutations can manifest with infantile lactic acidosis, childhood (cardio)myopathy and/or adult-onset PEO. Four patients with biallelic C1QBP mutations were reported with cardiomyopathy, but without involvement of the central nervous system [6]. In that small cohort, a boy (deceased at 18 days) had two compound heterozygous C1QBP variants (c.557G>C, p.Cys186Ser and c.612C>G, p.Phe204Leu), of which the latter affects the codon, deleted in our patient. In contrast, our patient had late-onset PEO with no overt cardiac symptoms. A recent study also reported two patients presenting with PEO-only and multiple mtDNA deletions without heart involvement, confirming our observation [7]. The adult female patient (54 years) was homozygous for the previously reported variant (c.612C>G, p.Phe204Leu). As the amino acid changes associated with early-onset cardiomyopathy are localized in important structural domains (e.g., beta strand of the protein) whereas variants found in PEO patients are localized in the coiled-coil region [7], the location of the variant could be important to explain or predict the different clinical manifestations.

The multiple mtDNA deletions in PEO patients harboring C1QBP mutations could be caused by the interaction between C1QBP and RNase H1. RNase H1 removes the replication template after one round of mtDNA replication, thus initiating
In conclusion, we identified a novel homozygous \textit{C1QBP} deletion in consanguineous patient of Indian descent, presenting with adult-onset PEO-plus and mtDNA multiple deletions. In the original paper [6], cardiomyopathy was always part of the clinical spectrum of \textit{C1QBP} pathogenic...
variants, but 2 studies [7, our paper] now report CIQBP pathogenic variants in patients without any heart involvement. This data indicates that CIQBP mutations have to be considered in patients with isolated PEO and PEO-plus phenotype.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2021.06.014.

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