

Identification of Novel Frameshift Variant in *PUS1* Gene by Whole Exome Sequencing

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Abstract

Introduction: Myopathy, lactic acidosis, and sideroblastic anemia 1 (MLASA1) is an autosomal recessive inheritance and a mitochondrial disorder associated with the *PUS1* gene. It is also associated with bone marrow and skeletal muscle and bone; its related phenotypes are high arc palate and myopathy. This study describes a 30-year-old woman with an MLASA1 phenotype.

Clinical report: The patient was diagnosed with developmental delay, progressive gait abnormality, and proximal and distal weakness in the lower limb. She also showed symptoms of bilateral facial paresia, lordosis, scapular winging, anemia, colored skin rash, and mild cognitive problems.

Methodology: Whole exome sequencing identified a novel variant in *PUS1*. Mutation c.365delG was then confirmed by PCR and sanger sequencing. The patient's parents' segregation analysis established the homozygosity and autosomal recessive inheritance pattern for c.365delG.

Results and Calculation: This study discusses the relationship between this novel mutation in *PUS1* and MLASA1 phenotype and its inheritance pattern in a consanguineous family.

Keywords

MLASA; PUS1; Lactic acidosis sideroblastic; Anemia

Introduction

Myopathy, lactic acidosis, and sideroblastic anemia (MLASA) is a mitochondrial respiratory chain disorder (MRCD) associated with mutations in PUS1, YARS2, and MTATP6. Exercise intolerance, muscle weakness, Micrognathia, Hypotonia, High arc palate, and Distichiasis, bone marrow, and skeletal muscle and bone are frequent symptoms of this disease. Delayed puberty, Glaucoma, Intellectual disability, and Microcephaly are among the less frequent ones [1]. MLASA1 (600462# OMIM) is associated with mutations in PUS1 gene, and MLASA2 (613561#OMIM) and MLASA3 (500011#OMIM) are respectively associated with mutations in the YARS2 gene (NM_001040436.2) at 12p11.21 and MTATP6 [2]. PUS1 encodes the nuclear pseudouridine synthase 1, which pseudouridinylates tRNA. Pseudouridine in tRNAs is involved in stopping codon suppression, translational specificity, and recoding efficiency [3]. Mutation in this gene could confront patients lacking pseudouridine in their tRNAs. Pseudo uridine affects the structure of tRNAs by affecting base- stacking in the anticodon loop. When it manifests in stems or the anticodon, it strengthens base-pairing. The stabilized conformation of the anticodon stem-loop could help to keep correct anticodon-codon pairings during translation. This stability increases translational accuracy by decreasing the rate of peptide bond formation and also lets incorrect codon-anticodon pairs have more times to be rejected [6] Table3. displays all the presented variants of PUS1 in HGMD. Mutation c.365delG is a likely pathogenic, homozygous variant with an autosomal recessive inheritance pattern located at chromosome 12 exon 3. (p.S123Qfs*12). This variant is a novel mutation that has not been reported to the HGMD, gnomAD, and clinVar databases. This study reports a novel mutation in *PUS1*, found by whole exome sequencing and convinced by PCR and sanger sequencing, and discusses the relation of this mutation with MLASA type1 (600462# OMIM) based on clinical reports and analyses.

Methods

The patient's whole exome sequencing (WES) data, with a coverage depth of 150x, was obtained from the patient's genomic DNA (gDNA). The gDNA was isolated from the patient's blood specimen using a filter-based methodology. After quality control and quantification, the DNA sample was prepared, and the Sequencing libraries were generated using Agilent Sure Select Human All ExonV7 kit. In the next step, 180- 280bp fragments were generated by aydro dynamic shearing system, modified, and enriched in a PCR reaction. After purifying and quantifying the products, the sequences were generated using NovaSeq 6000 Illumina sequencers. The following steps were performed on a Unix- based operating system. Fast QC and Illu QC tools were used for QC and trimming the sequencing data. Burrows-Wheeler Alignment Tool (BWA) was used for aligning the sequence reads to the GRCH37/UCSC hg19 human reference Genome. Latterly, Picard and GATK packages were applied for base quality score recalibration (BQSR). Then, variant calling and VQSR filtering were applied, and for the last step, the VCF file was annotated using ANNO- VAR, and the results were filtered to find the candidate variants. After checking

all the remaining variants in OMIM, the variants were classified based on the American College of Medical Genetics (ACMG) and Association for Molecular Pathology criteria (AMP) protocols. After choosing the candidate variant, DNA extraction, PCR, and Sanger sequencing were performed to confirm the validation of the selected mutation. The patient's genomic DNA was extracted using in- nuPREP Blood DNA Mini Kit anger, and Sanger sequencing was operated using an ABI prism 3730 sequencer.

Results

Clinical Report

Patient

This study involves a 30-year-old woman with consanguine parents who presented with progressive gait abnormality and proximal and distal weakness in the lower limb, followed by upper limbs from 6 years ago. She could not walk or crawl until 3 years old, and from 6 years ago, her muscular weakness grew. She cannot walk without help, has difficulty going upstairs, and currently uses a wheelchair. She has weak hands and only lifts lightweight objects. She has a positive Gower sign and mild MR. She had bilateral facial paresia, lordosis, scapular winging, anemia, colored skin rash, and mild cognitive problems and received blood at 4 months old. She had developmental delays. She does not have myalgia, cramps, osteoporosis, or cardiopulmonary. The paradigm tree of the proband has been represented in Figure 1.

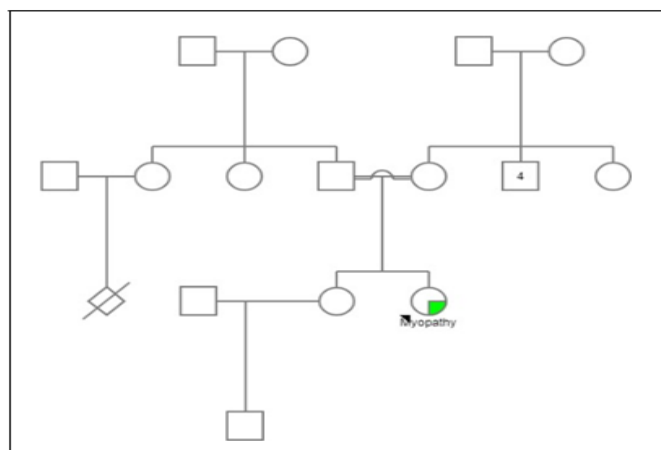


Figure 1: Pedigree of the family

Test and Results

Proband's brain MR study with multi-planner images (MRI) in different pulse sequences shows normal results. BMA and BMB reveal mild erythroid hyperplasia with no evidence of fibrosis. The muscle biopsy is within normal. Table 4 shows the patient's blood test reports after receiving blood. R.B.C level is lower, and the M.C.V and E.S.R factors and LDL levels are higher than the normal range. CPK levels is 33.5 and LDH is 26.8. EMG and NCV tests diagnosed myopathy Table 1.

Test Result Unit	Reference Interval
F.B.S 81 mg\dl	60 – 110
creatinine 0.6 mg\dl	F: 0.6 – 1.3
S.G.O.T(AST) 15 IU/L	up to 32
S.G.P.T(ALT) 16 IU/L	F :< 32
Alkaline Phosphatase 116 IU/L	61 – 306
L.D.H 238 U/L	< 180
Bilirubin total 0.9 mg\dl	0.2 – 1.2
Bilirubin Direct 0.1 mg\dl	0 – 0.3
Bilirubin indirect 0.80 mg\dl	
C.B.C	
W.B.C 3890 μ l	4000 – 10000
R.B.C 3.19 x106\mathmu L	4.25 – 5.4
Hemoglobin 10.5 g/dL	12.3 – 15.5
Hematocite 32.8 %	36 – 45
M.C.V 102.8 f L	80 – 98
M.C.H 32.9 pg	27 – 32
M.C.H.C 32.0 g/dL	33 – 38
R.D.W 21.9 %	11.6 – 14.6
M.P.V 9.6 f L	-
Platelets 252000 μ l	150000 – 450000
E.S.R 1 hrs 20 mm/h	Up to 15

Table 1: Patient's BloodTest.

Mutation Analysis

After performing the whole exome sequencing analysis that was previously elucidated in section.2,194857 annotated variants were identified. The annotated file was again filtered indifferent stages, and a myopathy panel was applied to initiate the most compatible variants with the proband's symptoms. Lastly, the homozygous variants were selected due to the con-sanguineous relationship of the patient's parents, so the number of filtered variants was lessened to a total of 21. The number of selected variants after each filtering step can be seen in Figure 2.

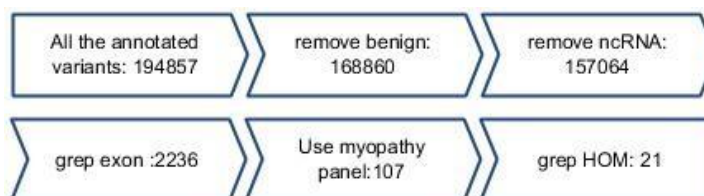


Figure 2: Variant filtering workflow.

All the variants were checked on OMIM and classified via ACMG/AMP protocols. This novel mutation (NM_025215: exon3:c.365delG:p.S123Qfs*12) was marked as likely pathogenic on the Var

Some databases. Because this mutation of the *PUS1* gene has a frameshift, it is marked as PVS1. Also, because it has not been reported to gnom AD, it has a PM2 label as well. This newly found mutation, located in exon 3 of *PUS1*, was then confirmed using PCR and sanger sequencing. To produce a 591-bp segment for PCR, the forward and Reverse primer sequences, GATTCCATTCCACTACCCACC and AAGCAAAGACCAGAGAGAACACAG, were designed by the Oligo7 software (Table 2). The PCR steps are as follows: (1) denaturation at 95°C for 5 minutes (Table 2).

	Sequence (5'→3')	Generator Tm	CG%
Forward primer	GATTCCATTCCACTACCCACC	60.94	52.17
Reverse primer	AAGCAAAGACCAGAGAGAACACAG	60.98	45.83
product length	591bp	-	-

Table 2: Primer pairs designed by the Oligo7 software.

(2) amplification by using 30 cycles at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds.

(3) Extension at 72°C for 5 minutes. The PCR's steps and protocols are represented in Figure 3. Finch TV was used for analyzing the DNA sequences. Figure 2-Figure 4 shows the patient's parents selected forward and reverse sequences (GTCCGCC-CTCGTCCG and TCAGGCTGTATTCTGAAA), which confirms the homozygosity for c. 365delG. Thus, it was concluded that this mutation has an autosomal recessive inheritance pattern.

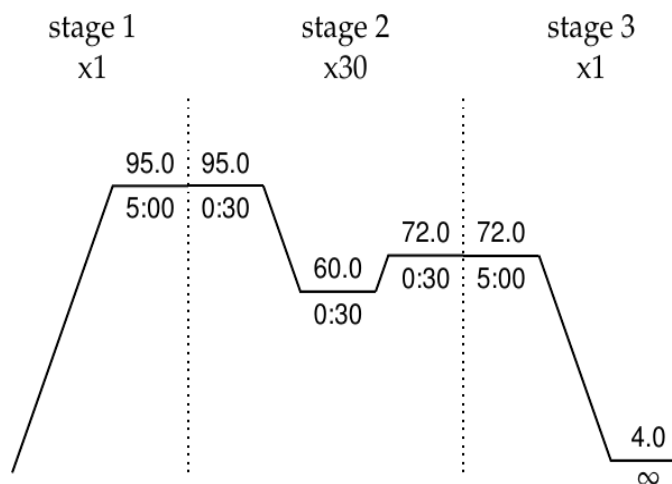


Figure 2: PCR'S steps and protocols.

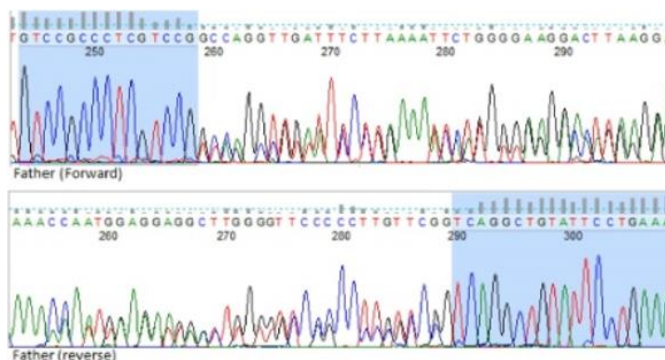


Figure 3: Patient's Father's sequences.

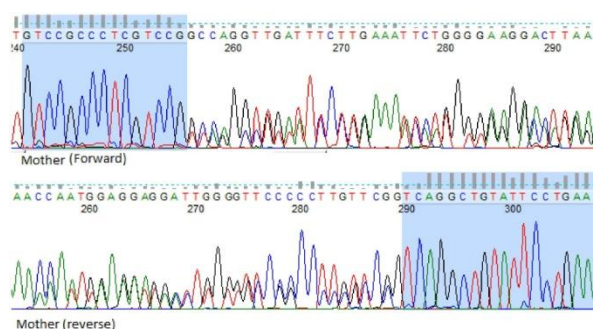


Figure 4: Patient's Mother's Sequence.

Discussion

The MLASA is caused by the absence of significant reduction of tRNA pseudouridylation. Pseudouridylation is a posttranscriptional modification and the most abundant modification in cellular RNA. Some of its roles in the cell are translation, localization, and stabilization of RNA. Transcription alters a variety of chemically distinct modifications of the RNA [9]. Pseudouridine is vital in anticodon stem-loop (ASL) and stabilizes the ASL's dynamic structure. Therefore, it makes the proper binding of tRNAs to the ribosome [10]. *PUS1* Converts specific uridines to PSI in several tRNA substrates and acts on positions 27/28 in the anticodon stem and positions 34 and 36 in the anticodon of an intron containing tRNA [11]. Pseudouridine causes anticodon codon pairings during translation to go well, increasing translational accuracy in general [12].

From this article, it can be concluded that the novel mutation of *PUS1*(p.Ser123GlnfsTer12) is associated with myopathy, lactic acidosis, and sideroblastic anemia 1 (MLASA1). This report discussed the case of a proband, a 30-year-old woman diagnosed with myopathy, progressive gait abnormality, proximal and distal weakness in the lower and upper limbs, and developmental delays. After performing WES, a novel mutation of the *PUS1* gene (p.Ser123GlnfsTer12) was found on her genomic DNA. This gene encodes a Pseudouridine synthesis involved in the Pseudouridylation process. This study is the first report of this novel mutation in the *PUS1* gene, and it shows that the *PUS1* is responsible for MLASA1, and this result is compatible with the tests and the patient's clinical reports.

Authors Contribution

Data curation: Sajad Rafiee Komachali. Clinical report analysis: Farid Momeni, Mohamad Javad Maleknia, Firooze Ronassian; Methodolgy: Romina Maleknia, Sajad Rafiee Komachali; Writing original draft: Romina Maleknia, Farid Momeni; Writing review & editing: Romina Maleknia, Mohamad Javad Maleknia, Sajad Rafiee Komachali; Funding acquisition: Mansoor Salehi.

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Ethical Approval

All the patients gave their informed consent for inclusion in this research.

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