Identification of a Novel *MICU1* Nonsense Variant Causes Myopathy with Extrapyramidal Signs in an Iranian Consanguineous Family

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Case report

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Abstract

Background: Ca^{2+} as a universal second messenger regulates basic biological functions including cell cycle, cell proliferation, cell differentiation and cell death. Lack of the protein mitochondrial calcium uptake1 (MICU1) which has been regarded as a gatekeeper of Ca ions, leads to the abnormal mitochondrial Ca^{2+} handling, excessive production of reactive oxygen species (ROS), and increased cell death. Mutations in *MICU1* gene (NM_006077.3) causes a very rare neuromuscular disease, Myopathy with extrapyramidal signs (MPXPS), due to primary alterations in mitochondrial calcium signaling which demonstrates the key role of mitochondrial Ca^{2+} uptake. To date 6 variants have been reported in *MICU1* gene in approximately 21 pedigrees.

Case presentation: here we report a 44-year-old Iranian patient presented with learning disability, muscle weakness, easy fatigability, reduced tendon reflexes, ataxia, elevated hepatic transaminase, elevated serum creatine phosphokinase and gait disturbance. We Identified a novel nonsense variant c.385C>T; p.(R129*) in *MICU1* gene by whole exome sequencing and segregation analysis.

Conclusions: Our finding along with previous studies provides more evidence on the clinical presentation of the disease caused by pathogenic mutations in *MICU1*. Finding more variants and expanding the spectrum of the disease increases the diagnostic rate of molecular testing in screening of this kind of diseases and in turn improves the quality of counseling for at risk couples and helps them to minimize the risks of having affected children.

Background

Mitochondrial Ca^{2+} uptake which has been long established as a key mediator of cell survival, metabolism and death needs to be tightly regulated (1, 2). The predominant mechanism among ion transporters capable of Ca^{2+} uptake into mitochondria is through a highly Ca^{2+} -selective ion channel located in the inner membrane called the Mitochondrial Calcium Uniporter (MCU), driven by electrochemical gradient across the inner mitochondrial membrane (IMM) (3–6). Mitochondrial Calcium Uptake 1 (MICU1), a regulatory subunit that shields mitochondria from Ca^{2+} overload, is required for uniporter-mediated Ca^{2+} uptake (7). MICU1 is an E hand protein and has been suggested as a Ca^{2+} sensor which sets the threshold of extramitochondrial Ca^{2+} load for mitochondrial Ca^{2+} uptake (8, 9). MICU1 acts as both of activator and inhibitor of mitochondrial calcium uptake depending on the concentration of calcium. As a gatekeeper of MCU at low Ca^{2+} levels MCU1 prevents channel opening and at high Ca^{2+} levels promotes MCU opening which allows rapid response of mitochondria to calcium signals generated in the cytoplasm (2, 10, 11). Although Ca^{2+} influx to mitochondrial matrix is crucial for the life of a cell and its vital functions, Mitochondrial Ca^{2+} uptake also drives physical mitochondrial damages leading to bioenergetics crises and programmed cell death under conditions of cellular Ca^{2+} overload (12, 13). In other word MCU translates cellular Ca^{2+} signals into metabolic or death responses (14). Thus, mitochondrial Ca^{2+} uptake must be strongly regulated which is achieved by sensing the

cytosolic calcium ions fluctuations and balancing the rates of Ca^{2+} uptake and release (15). Several lines of evidence link mitochondrial Ca^{2+} overload to a range of pathological conditions including cancers, diabetes, cardiovascular and neurodegenerative disorders (12, 16).

Abnormal mitochondrial Ca^{2+} handling due to biallelic *MICU1* variants had been shown to cause the Myopathy with extrapyramidal signs (MPXPS), characterized by proximal myopathy, learning difficulties and a progressive extrapyramidal movement disorder (17).

Previous cell studies showed that in *MICU1*-mutant fibroblast cells compared to controls, the mitochondrial Ca^{2+} uptake was significantly elevated, as a consequence cytosolic Ca^{2+} signals were reduced, which can result in Ca^{+2}-dependent processes including synaptic transmission and muscle contraction disturbance. Also the overload in mitochondrial Ca^{2+} levels seen in mutant *MICU1* cells result in severe mitochondrial fragmentation and damage (15, 17).

In vivo molecular removal of *MICU1* leads to larval lethality in Drosophila (12). Whole body knockout of *MICU1* in the mouse has been shown to cause a high probability of perinatal lethality and the survived mice have physical biochemical abnormalities, ataxia and muscle weakness, recapitulating the problems observed in the human patients (18). Consistent with the clinical features displayed by patients, *MICU1* has been indicated to be highly expressed in normal mouse muscle and brain (17).

Dysregulation of *MICU1* in skeletal muscle fibers has been shown to result in sarcolemma, less contractile force, increased fatigue and diminished capacity to repair damage to their cell membranes. In accordance with problems identified in patients, the experimental model studies characterized more pronounced muscle weakness, and greater loss of muscle mass in certain muscles (19).

Loss of function variants in *MICU1*, which disturb Ca^{2+} signaling cause a very rare neuronal and muscular disorder characterized by impaired cognition, early muscle weakness, elevated creatine kinase in their bloodstream and an extrapyramidal movement disorder (17, 19).

Previous studies have reported recessive mutations in *MICU1* gene in cases presented with brain and muscle disorders. Logan et al. (2013) have identified two different homozygous mutations affecting a splice acceptor site, c.1078-1G > C and a splice donor site, c.741 + 1G > A in total of 15 UK-Pakestani and Dutch Caucasian patients respectively which suggested a common ancestral origin for individuals carrying each of the two variants (17).

Lewis-Smith et al. (2016) have identified a homozygous deletion of exon 1 of *MICU1* in a 9-year-old girl and her cousin (20). Sara Musa et al. (2018) reported a Middle Eastern founder nonsense mutation, c.553C > T (p. Q185*), in a consanguineous Middle Eastern Arab families. They investigated 13 patients from 10 families, of these 12 patients carried the p.Q185* variant in homozygous state while one patient was compound heterozygous for this founder mutation and an intragenic duplication of exons 9 and 10 (21). A novel mutation ,c.1295delA, in exon 13 of *MICU1* gene as the first report of MPXPS in Iranian population has been reported recently by Mojbafan et al. (2020) (22).

In accordance with previous studies, we identified a novel nonsense mutation c.385C > T p.(R129*) in *MICU1* gene, which is predicted to lead to a complete loss of function of *MICU1* in an Iranian patient with learning disability, muscle weakness, easy fatigability, reduced tendon reflexes, Ataxia, elevated hepatic transaminase, elevated serum creatine phosphokinase and gait disturbance.

Clinical Presentation

A 44-year-old man with a neurodegenerative disorder was referred to the Department of Medical Genetics, DeNA Laboratory, Tehran, Iran for genetic testing. His clinical symptoms were learning disability, muscle weakness, easy fatigability, reduced tendon reflexes, ataxia, elevated hepatic transaminase, elevated serum creatine phosphokinase and gait disturbance. His parents were first cousins and they were from North of Iran. Further genetic counseling revealed history of 2 other affected brothers in this family who died at the age of 46 and 48 years respectively, one of them due to heart failure and the other due to progressive symptoms of the disease; however no detail medical records were available for them. The parents claimed that they had similar symptoms with the proband.

For more detailed evaluations physical examination, muscle tissue biopsy and an electromyography and nerve condition velocity (EMG/NCV) test were performed. The physical examination of the affected proband showed raised CPK up to 2081 U/L (normal males: 24–195), LDH to 571 U/L (normal: 0-480) S.G.P.T (ALT) to 83 U/L (normal: 0–41) and S.G.O.T (AST) to 52 (normal: 0–37).

Muscle biopsy from right biceps showed myopathic atrophy with dystrophic features. Multiple necrotic/regenerative fibers, myophagocytosis and severe endomysial fibrosis were noted (Fig. 1a). Reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) staining revealed intermyofibrillar network disruption as moth-eaten fibers and core-like lesions (Fig. 1b). Adenosine triphophatase staining showed predominance of type 1 fibers and atrophy. The above histochemical pathologic findings were suggestive of muscular dystrophy, so immunohistochemical (IHC) study of dystrophin, sarcoglycans, merosin, beta-Spectrin and dysferlin proteins was performed and sarcolemmal labelling with all the above examined antibodies was observed. EMG/NCV study revealed short duration MUAPs in two upper and lower extremities tested muscles which was in favor of myopathic changes.

Eventually WES revealed a novel nonsense homozygous variant, c.385C > T; p.(R129*), which creates a premature stop codon in the MICU1. The homozygote normal and heterozygote state for this variant in the unaffected sister and his parents were confirmed by Sanger sequencing. The identified variant was classified as likely pathogenic (class 2) based on the American College of Medical Genetics (ACMG; http://wintervar.wglab.org/) criterion. Additionally, the consequence of this variant was predicted to be disease-causing by mutation tasting and damaging by SIFT. The variant was not present in Exome Aggregation Consortium (ExAC), 1000G, and Iranome databases, leaving it as a novel variant. Additionally, we showed that the affected amino acids are conserved between different species (Fig. 2).

Materials And Methods

Ethical consideration

The study protocol was approved by the local medical ethics committee of DeNA laboratory, Tehran, Iran. Following genetic counseling and informed consent for genetic testing, we obtained peripheral blood samples from the patient and his available family members. The authors declare that they have conducted the project ethically in accordance with the World Medical Association Declaration of Helsinki.

DNA Extraction

Genomic DNAs were extracted from the peripheral blood of the patients and all available family members by the High Pure PCR template preparation kit (Roche: Product No. 11814770001).

Targeted Next-Generation Sequencing

Whole exome sequencing (WES) was performed on affected individual (IV-3). Agilent's SureSelect Human All Exon V6 kit was used to enrich approximately 60 Mb of the Human Exome from fragmented genomic DNA. The generated library was sequenced on an Illumina Hiseq 4000 platform to obtain an average coverage depth of 100. Typically, 97% of the targeted bases were covered > 10. An end to end in-house bioinformatics pipeline including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering of low quality reads and probable artifacts, and subsequent annotation of variants, was applied. Reads were mapped to the reference human genome using the Burrows-Wheeler Aligner (http://bio-bwa.sourceforge.net/). Single-nucleotide variants (SNVs) and micro insertions-deletions (indels) were called using SAMtools (http://samtools.sourceforge.net/), based on filtered variants with a mapping quality score of > 20, and were annotated using ANNOVAR (http://www.openbioinformatics.org/annovar/).

Evaluation was focused on coding exons along with flanking ± 20 intronic bases. All disease causing variants reported in Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) as well as all variants with minor allele frequency (MAF) of less than 1% in publicly available mutation and polymorphism databases such as 1000 genome project (http://www.1000genomes.org/), ExAC (http://exac.broadinstitute.org/), Exome Sequencing Project (ESP) (http://evs.gs.washington.edu/EVS/) and gnomAD database (https://gnomad.broadinstitute.org/) were considered. We ended up with only one novel variant, c.385C > T, in *MICU1* gene. Prediction of the consequence of the c.385C > T; p.(R129*) was obtained from online databases namely SIFT (https://sift.bii.a-star.edu.sg/), and MutationTaster (http://www.mutationtaster.org/). For further consideration, the frequency of the variants was checked out on the local database, Iranome (http://www.iranome.ir/). Also, ConSurf (http://www.consurf.tau.ac.il) was applied to check the evolutionary conservation in the region of the variant (Fig. 2).

Segregation analysis

Segregation analysis was investigated in the family. For this purpose, primers surrounding region of the identified variant were designed using Primer3Plus (https://primer3plus.com/cgi-

bin/dev/primer3plus.cgi) web-based server (PCR conditions and primer sequences are available upon request). Consequently, DNA sequencing of the PCR products was performed on ABI 3130 with the ABI PRISM BigDye Terminator v. 3.1 sequencing kit (Applied Biosystems, USA). Sequencing chromatograms (Fig. 2) were analyzed using CodonCode Aligner software version 8.0.2 (CodonCode Corp, USA).

Sanger Sequencing confirmed the variant and its segregation with the disease in the family (Fig. 2).

In silico structural modeling

A comparative modeling was performed by utilizing combination of multiple templates and iterative optimization of alternative alignments for prediction of three dimensional structure of truncated protein. The I-TASSER server was implemented to predict MICU1 truncated (23). The crystal and three dimensional structure of MICU1 protein (PDB ID: 4nsc) was applied as a template. Predicted structures were scored by QMEAN server (http://swissmodel.expasy.org/qmean/) (24, 25). To optimize the truncated structure, YASARA Energy Minimization Server was implemented. After recruiting the structure in a distinct PDB file, the protein structure and possible impact of the novel variant on protein flexibility and stability were depicted by Chimera 1.13 and molegro vitual docker (26). A schematic pattern of wild and truncated protein was drawn using IBS software (Fig. 3a) (27).

Discussion And Conclusion

Whole-exome sequencing of the affected individual identified a likely pathogenic variant in homozygous state, c.358C > T in exon 4 of *MICU1* gene. Pathogenic variants in the *MICU1* gene which is located on 10q22.1 chromosome region have been shown to be associated with proximal muscle weakness and learning disabilities due to deregulated mitochondrial Ca^{2+} uptake, resulting in mitochondrial Ca^{2+} overload, excessive production of ROS, and increased cell death.

Mitochondrial function is crucial for energy provision especially in excitable cells including skeletal and cardiac muscle cells, neurons, and glia cells and protects them from damage via fluctuation of Ca^{2+} (18).

Ca^{2+}, a versatile and ubiquitous intracellular messenger (28), plays a central role in a remarkably wide range of cellular processes especially in nervous system and muscle. Calcium ions have been implicated to mediate neuronal gene expression, neuronal development and plasticity, synaptic transmission, neurotransmitter release, neuronal excitability, data processing, cognition, learning and memory in brain; excitation-contraction coupling, energy metabolism, adaptation to exercise and sarcolemmal repair in muscles (19, 29–31).

Ca^{2+} uptake regulation in the mitochondria is vital to control crucial functions like ATP production or cell death. This regulation in the outer membrane is mediated through the voltage-dependent anion selective channel (VDACs) and in the inner membrane occurs through channels such as Mitochondrial Calcium Uniporter (MCU) which mediates mitochondrial Ca^{2+} uptake through its regulators, MICU1 and MICU2 (2).

In the inner mitochondrial membrane (IMM), there is a mitochondrial calcium uniporter complex, containing four subunits: MCU, MICU1, MICU2, and EMRE. Ca^{2+} selectivity contributed by the MCU. MICU1–MICU2 are essential for Ca^{2+} uptake and EMRE is required for Ca^{2+} infiltration. Loss of MICU1–MICU2 results in Ca^{2+} leakage and interrupts respiration dependent to ATP synthesis which causes brain and muscle disorders in humans (14).

The MCU has been shown to mediate mitochondrial Ca^{2+} uptake which needs the mitochondrial calcium uptake 1 (MICU1) protein as a crucial element for its regulation. MCU is a channel that does not have much affinity for Ca^{2+}, thus it uses different channels and proteins such as MICU1 which bind to Ca^{2+}, to be activated. It has been shown that down-regulation of MICU1 suppresses mitochondrial Ca^{2+} significantly.

MICU1, which is localized to the mitochondrial inner membrane, is a ~ 54-kDa protein consists of 476 amino acids. It contains two parts including a transmembrane helix (aa ~ 33–52) and a cytosolic C-terminus (aa ~ 53–476) which contains two EF-hand Ca^{2+}-binding domains (EF1 and EF4) which help activating MCU.

The structure of Ca^{2+}-free MICU1-xtal is shown to contain four regions, the N-domain, including three a-helices and three antiparallel b-strands, the N-lobe and the C-lobe including six and seven a-helices respectively and the C-helix which plays an important role in calcium uptake in the mitochondria by mediating binding of MICU1 to MCU and formation of a ~ 480 kDa complex and oligomerization in the presence of Ca^{2+} (32). (Fig. 3b)

As MICU1 has an essential role in mitochondrial Ca^{2+} uptake, defects in this protein results in increasing Ca^{2+} level in mitochondria and reduction of cytosolic Ca^{2+} which facilitates apoptosis process, learning anomalies, muscle weakness due to difficulties in myofiber regeneration, and extra-pyramidal movement disorder. Studies showed that MICU1 plays a gatekeeper role in interaction with MCU (33).

Also it has been shown that different neuromuscular diseases are caused due to mutations in *MICU1* which lead to the deregulation of cytoplasmic and mitochondrial Ca^{2+} levels and interrupting Ca^{2+}-dependent processes such as muscle contraction and synaptic transmission (17).

MICU1 consists of 6 chains. The nonsense variant found in this study affects the exon 4 of this gene and therefore affects 5 of these chains. The structural analysis revealed that EF-hand motif, which has an important role in transferring Ca^{2+} through mitochondrial membrane (34), begins from amino acid 218, thus the R129* mutation disrupts this motif as well as the structure and function of MICU1 protein and leads to a complete loss of function of MICU1 protein. Figure 3b shows the topological structure of wild type and mutant form of MICU1 protein.

According to the ACMG-CAP guideline (35): 1) Nonsense variant in *MICU1* gene, which leads to loss-of-function, is associated with myopathy and is a known mechanism of disease. (PVS1). 2) Pattern of

inheritance is found to be autosomal recessive (PM3). 3) Co-segregation with the disease as heterozygous carriers are not affected while the homozygous individual shows myopathy phenotype. In addition it was not found in ethnically matched healthy controls, Iranome (PS4). 4) This variant was not found in Human Gene Mutation Database (HGMD), ClinVar, 1000 genome project, Exome Aggregation Consortium (ExAC) Exome Sequencing Project (ESP), and gnomAD database (PM2). 5) Pathogenic computational verdict based on 5 pathogenic predictions from BayesDel_addAF, DANN, EIGEN, FATHMM-MKL and MutationTaster vs no benign predictions (PP3). According to ACMG-CAP rules for combining criteria to classify sequence variants (PVS1 + PM3 + PS4 + PM2 + PP3), this variant is classified as pathogenic.

In this study, we reported a novel nonsense variant in the *MICU1* gene in an Iranian patient with extrapyramidal signs. This is the second report of MPXPS from Iran.

Accurate identification of causative mutations in families play a key role to provide them with appropriate genetic counseling, Pre-implantation Genetic Diagnosis (PGD), Prenatal Diagnosis (PND), management and/or further therapy strategies.

Abbreviations

MICU1: mitochondrial calcium uptake1; ROS: reactive oxygen species; MPXPS: Myopathy with extrapyramidal signs; WES: Whole Exome Sequencing; MCU: Mitochondrial Calcium Uniporter; IMM: inner mitochondrial membrane; EMG/NCV: electromyography and nerve condition velocity; NADH-TR: nicotinamide adenine dinucleotide tetrazolium reductase; IHC: immunohistochemical; ACMG: American College of Medical Genetics; ExAC: Exome Aggregation Consortium; SNVs: Single-nucleotide variants; HGMD: Human Gene Mutation Database; MAF: minor allele frequency; ESP: Exome Sequencing Project; VDACs; voltage-dependent anion selective channel; PGD: Preimplantation Genetic Diagnosis; PND: Prenatal Diagnosis.

Declarations

Acknowledges

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Authors' contributions

M.G, and F.B conceived and designed the experiments; F.B, and M.Kh conducted the experiments; M.G, and F.B analyzed and interpreted the data; F.B, M.Kh, and F.ZD wrote the paper; M.G revised the draft critically for important intellectual content; E.AS and N.A collect the detailed information and blood samples of pedigree; Y.N performed the immunohistochemistry study, analysis and interpretation of data. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data supporting the conclusion of this manuscript will be made available by the corresponding author.

Ethics approval and consent to participate

The authors declare that they have conducted the project ethically in accordance with the World Medical Association Declaration of Helsinki. The written, informed consent was obtained from all participants or their respective guardians. Participants also provided the written informed consent for publication of their related information included in this paper.

Consent for publication

Written informed consent was provided for publication of all the available participants included in this paper.

Competing interests

The authors declare that they have no competing interests.

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Figures

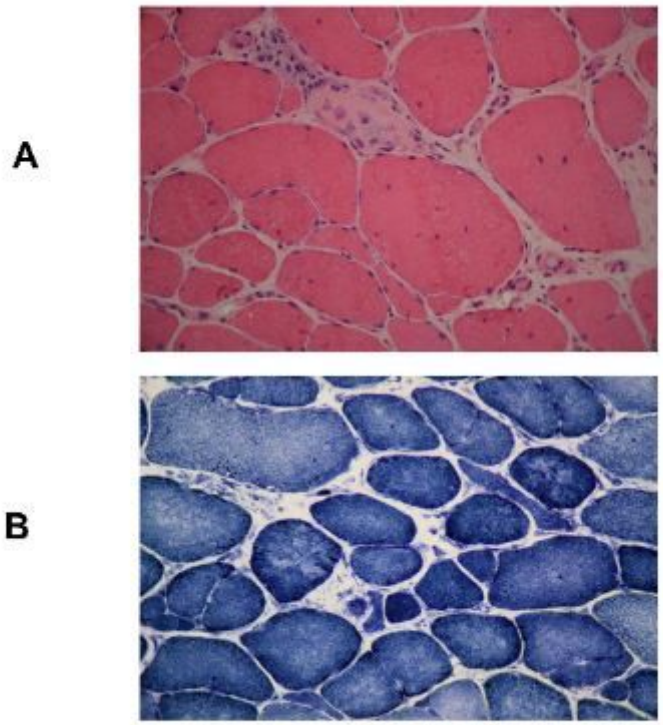

Figure 1

A) Prominent fibers size variation with necrosis and myophagocytosis associated with severe endomysial fibrosis, fiber splitting and increased internalization of nuclei (Hematoxylin and eosin x400). B)

Intermyofibrillar network pattern is disrupted with presence of core-like lesions (NADH-TR x400).

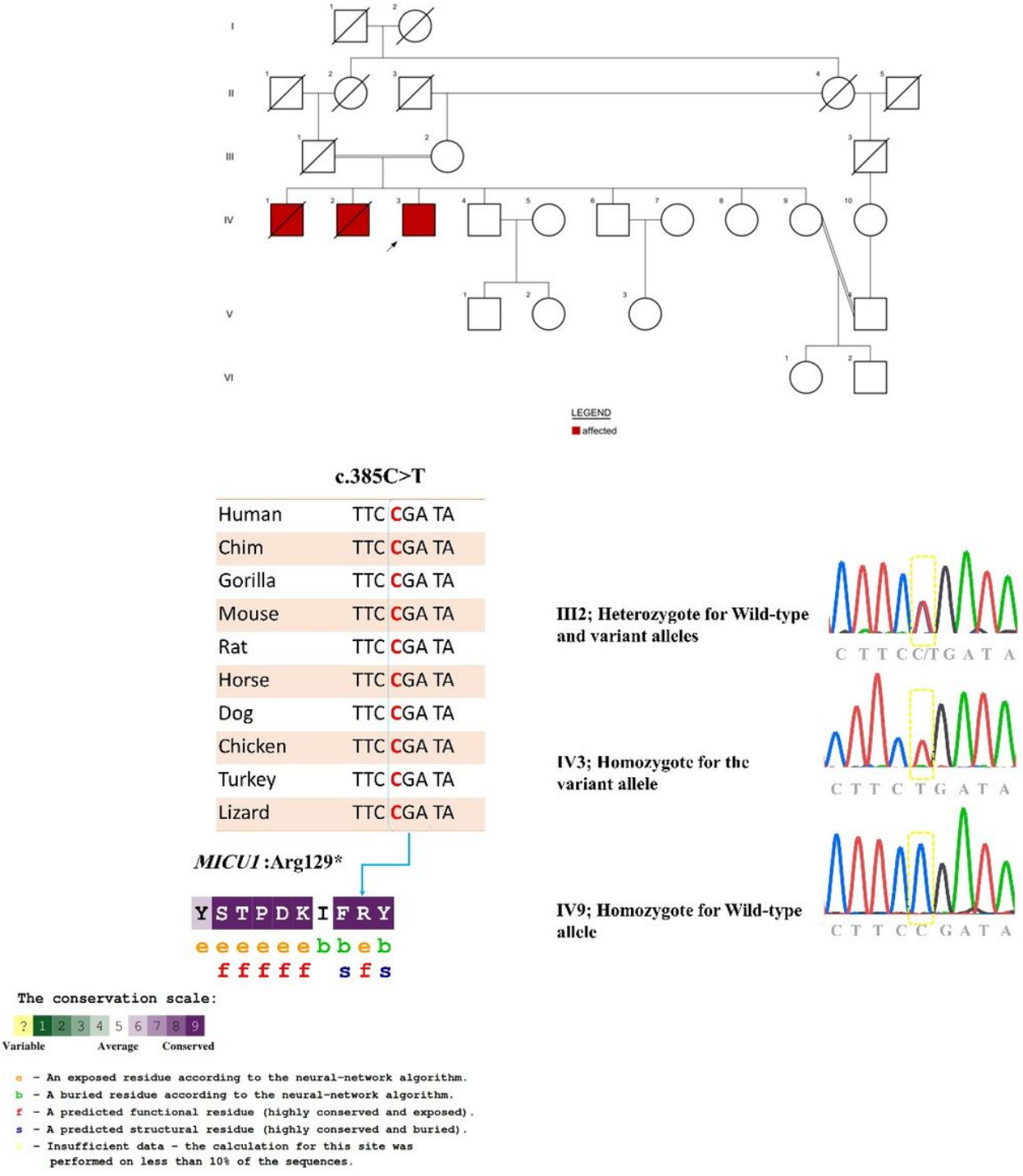

Figure 2

Representative pedigree, Sequence chromatograms confirming the mutation, Cross-species alignment, and ConSurf result of amino acid

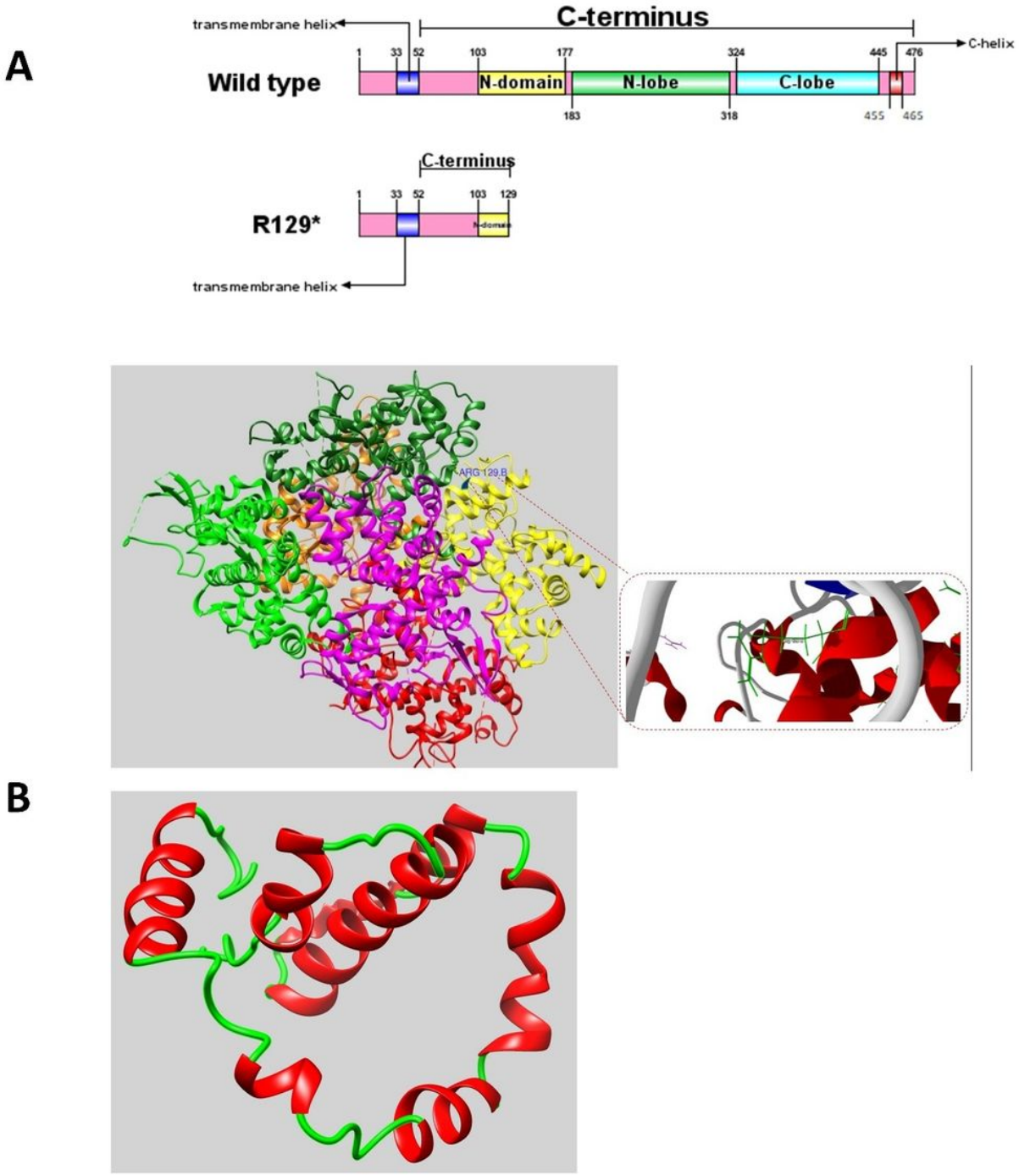

Figure 3

A) Schematic comparison of wild type and mutant predicted MICU1 structures. The mutated site of p. (Arg129 to stop codon) is highlighted by a blue zone and locally zoomed. This nonsense mutation removed the functional chains of MICU1 protein that contribute in EF-hand structure B) Structure modeling of mutant type was based on a well-known template (PDB: 4nsc).