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Case Report

# Homozygous Mutation in *TWNK* Cases Ataxia, Sensorineural Hearing Loss and Optic Nerve Atrophy

**Faezeh Jamali, MSc[1]; Hamid Ghaedi, PhD[1]; Abbas Tafakhori, MD[2]; Elham Alehabib, PhD[3]; Marjan Chapi, MSc[1]; Narsis Daftarian, MD[4]; Hossein Darvish, PhD[5*]; Javad Jamshidi, MSc[6,7]**

[1]Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Iranian Center of Neurological Research, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran
[3]Student Research Committee, Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Ocular Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5]Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran
[6]Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran
[7]Neuroscience Research Australia, Sydney, NSW, Australia

**Abstract**

The *TWNK* (*C10orf2*) gene encodes Twinkle, an essential helicase for mtDNA replication. Homozygous mutations in *TWNK* can lead to mitochondrial DNA depletion syndrome 7 (MTDPS7) that usually manifests as Infantile onset spinocerebellar ataxia (IOSCA). Here, we report a 15-year-old Iranian boy with three main symptoms; ataxia, sensorineural hearing loss and optic nerves atrophy which were accompanied by other symptoms including flexion contracture, dysarthric speech, nystagmus, dystonia and borderline intellectual disability. Whole exome sequencing (WES) revealed a homozygous mutation in his *TWNK* gene. The mutation was a transversion which replaced a C with A (NM_021830.4 (TWNK):c.874C>A). This nucleotide substitution results in replacing a Threonine with Proline in codon 292 of Twinkle protein (p.Pro292Thr). *In silico* analyses showed that this amino acid change in Twinkle could be deleterious and disease-causing; therefore, we attribute the symptoms of our patient to this mutation. Our study extended the homozygous mutation spectrum of the *TWNK* gene that leads to IOSCA.

**Keywords:** *C10orf2*, Hearing loss, Spinocerebellar ataxia, Infantile, Iran, Optic neuropathy

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## Introduction

Mutations in genes related to mitochondrial functions cause a variety of human disorders and many of them mainly affect the nervous system, a high energy-demanding organ.[1] The *TWNK* (OMIM# 606075) gene encodes Twinkle protein, a hexametric ring DNA helicase that, along with mitochondrial single-stranded DNA binding protein and mtDNA polymerase gamma, have a key role in mtDNA replication.[2] Mutations in *TWNK* can cause a variety of symptoms; however, three conditions have been linked to *TWNK* mutations: Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant 3 (PEOA3) (MIM# 609286),[3] Mitochondrial DNA depletion syndrome 7 (MTDPS7) (MIM# 271245)[4] and Perrault Syndrome 5 (PRLTS5) (MIM# 616138).[5] PEOA3 is caused by heterozygous mutations in *TWNK* while PRLTS5 and MTDPS7 are the result of homozygous mutations. MTDPS7 which usually manifests as infantile onset spinocerebellar ataxia (IOSCA) shares some features with PRLTS5 but is a more severe disorder. The main symptoms of MTDPS7 include hypotonia, infantile or adult-onset spinocerebellar ataxia, athetosis, ophthalmoplegia, hearing deficit, seizures, and sensory neuropathy, although there is considerable phenotype variability in patients.[6] Here, we present an Iranian boy with a missense mutation in the *TWNK* gene and symptoms which are closest to patients with IOSCA.

## Case Report

The patient was a 15-year-old boy from Mazandaran province, northern Iran. He was the first and only child of consanguineous parents. He was born following an uneventful pregnancy with a birth weight of 3.5 kg (Z-score: 0.31). His problems started from the first year of his life with delayed speech and standing. At the age of four, the patient was able to communicate verbally but his talking was inaccurate from the beginning. At this time, his parents noticed his difficulty in hearing; auditory tests (pure tone audiometry and ABR) detected almost complete hearing loss in both ears and hearing aid was fitted to the patient.

From the age of eight, his symptoms got worse and his eyesight was disrupted, forcing him to use glasses since that time. His eyesight gradually worsened and now, the best corrected visual acuity is 20/200 in both eyes with bilateral optic atrophy. In addition, the patient gradually

***Corresponding Author:** Hossein Darvish, PhD; Department of Medical Genetics, Semnan University of Medical Sciences, Semnan, 1438814567, Iran. Tel: 09111783431; Email: darvish_mg@yahoo.com, darvish_mg@sbmu.ac.ir

suffered a balance and ataxia disorder so that his walking was slowly affected. Now, he is only able to walk with help because of severe ataxia. The patient's fingers have flexion contracture in both hands (Figure 1) and abnormal movement (dystonia) is also seen in his hands. His brain MRI was normal but electromyography (EMG) and nerve conduction velocity (NCV) tests showed bilateral upper and lower chronic axonal sensory and motor polyneuropathy. The patient has never had any seizure. He has a score of 66–76 on the Raven Intelligence Scale and a score of 79 on the Vineland social maturity scale, suggestive of borderline intellectual disability.

### Genetic Analysis

DNA was extracted from the patient's and his parent's peripheral blood and whole exome sequencing (WES) was performed for the patient. Whole-exome capture and next-generation sequencing were performed by the Macrogen Company (Seoul, South Korea).

To confirm the results of WES, a pair of primers were designed to amplify a short sequence of the location of the detected mutation using PCR for further Sanger sequencing. The WES result, which was then confirmed by Sanger sequencing, revealed a homozygous missense mutation in exon 1 of the *TWNK* gene (NM_021830.4(TWNK):c.874C>A). Both of the patient's parents were heterozygous for the same mutation (Figure 2).

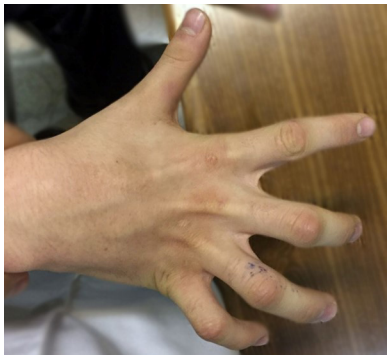

**Figure 1.** Flexion Contracture in The Patient's Hand.

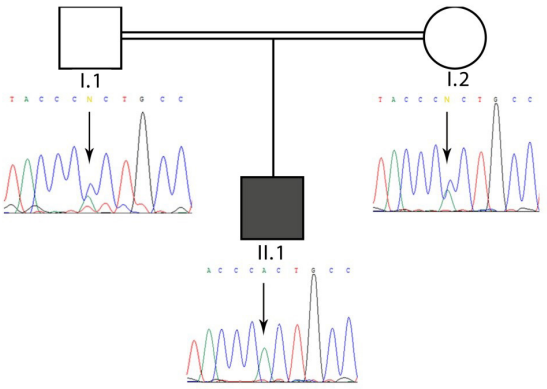


**Figure 2**. Family Pedigree and Partial Sequence of *TWNK* Eexon 1, the Location of the Mutation. The arrow above the partial sequence of *TWNK* is the site of mutation.

We searched in the human gene mutation database (HGMD), dbSNP, ExAC, and a local database including 800 Iranian samples (http://www.iranome.com) to exclude the normal variations and confirm the novelty of the mutation.

The mutation was annotated with PolyPhen-2, MutationTaster, Grantham score, CAAD score and UMD-predictor to assess the putative variant effect on the Twinkle protein. The structural model of the Twinkle protein was predicted with Phyre2 webserver and the HOPE project (http://www.cmbi.ru.nl/hope) and PyMol were used to analyze and visualize the mutation effects. The Proline to Threonine change was estimated to be deleterious and disease-causing by different tools (Table 1).

It was predicted that the wild-type residue resided in the Twinkle, Toprim Domain (InterPro ID: IPR034154) with topoisomerase-primase nucleotidyl transferase/hydrolase forms functions. As this is a conserved domain, its mutations will have deleterious consequences. Figure 3 provides a graphical representation of the mutation modeled on Twinkle protein.

## Discussion

The three main symptoms of our patient were ataxia, sensorineural hearing loss and optic nerve atrophy which were accompanied by other symptoms including flexion contracture, dysarthric speech, nystagmus, dystonia and borderline intellectual disability.

Mutations in the *TWNK* gene causing human diseases were first reported by Spelbrink *et al*. by finding two different heterozygous mutations in two families with PEOA3.[7] Then, it was shown that recessive mutations in *TWNK* can lead to a more severe neurodegenerative disorder MTDPS7, manifesting as IOSCA.[4] It can be said that PRLTS5 is a milder form of MTDPS7 which also manifests as a recessive disorder. Most of *TWNK* mutations reported to date are dominant and cause PEOA3.[6]

Variable expression is part of conditions caused by recessive mutations in *TWNK*. The difference in severity and symptoms of patients with homozygous or compound heterozygous mutations in *TWNK* can be attributed to the environmental factors, other modifier genes and genetic background, or the type and location of the mutation in the gene, although a clear correlation between the location of mutations in *TWNK* and the symptoms has not been described yet.

The Twinkle protein has three main domains: the primase domain, the linker region and the helicase domain.[8] The primase, the linker and a part of the helicase domain are in exon 1, and exons 2 to 4 code for the rest of the helicase domain. Our patient had an amino acid change (Pro to Thr) in codon 292 of the protein which is located in the

**Table 1.** Annotation of NM_021830.4(TWNK):p.(Pro292Thr) Mutation with Predictive Tools

| UMD-Predictor (Score, prediction) | CAAD Score | Grantham Score | Mutation Taster (Score, prediction) | PolyPhen (Score, prediction) | gnomAD exomes allele frequencies | Allele: frequency (count) | Alleles (ref, alt) | Position (hg38) |
|---|---|---|---|---|---|---|---|---|
| 93, Pathogenic | 22.9 | 38 | 38, disease causing | 0.95, probably damaging | C: 100% | C: 0.99999593235 (245841) | C>A | Chr10: 100989084 |

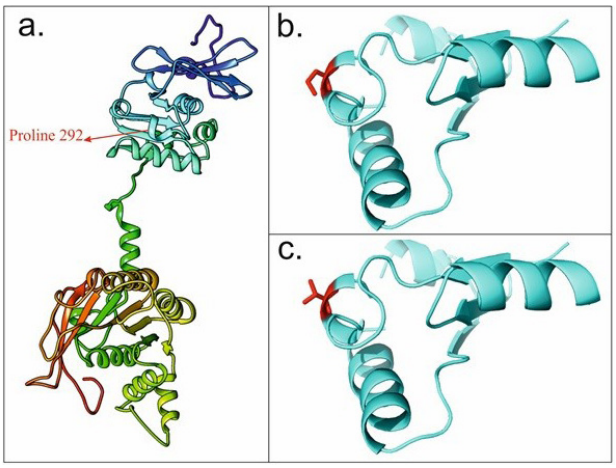


**Figure 3.** Overview of the Twinkle Protein Structure and Mutation. (**a**) The twinkle protein in ribbon-presentation, (**b**) The wild type Proline at 292 residue and (**c**) mutant threonine residue on closer view. The color red is used to indicate the mutation position in the structure. This figure is generated by homology modeling method as implemented in Phyr2 server.

primase domain. *In silico* analysis revealed that this amino acid change possibly disrupts the protein structure and may therefore have a pathogenic effect (Table 1).

IOSCA due to mutations in Twinkle was first described by Nikali et al in Finland by reporting a homozygous founder mutation (Y508C) in the helicase domain of the gene.[4] In addition to homozygous mutations in the helicase domain, homozygous mutations in the primase domain[9] and compound heterozygote mutations in the primase and helicase domains[10] have also been reported in patients with IOSCA. There is no report of mutations in the linker domain associated with this condition.

As patients with *TWNK* mutations are rare and there is no clear phenotype-genotype coloration between homozygous mutations of *TWNK* and the severity of symptoms, individual reports of mutations alongside clinical manifestations would be helpful in estimating the prognosis of the disease for a specific mutation. Our study extended the homozygous mutation spectrum of the *TWNK* gene that leads to IOSCA.

### Authors' Contribution

FJ, EA and MC collected the patient's data and conducted the laboratory experiments. AT and ND examined the patient and helped with clinical data. HG investigated the mutation consequences using bioinformatic tools. HD and JJ Conceived and designed the analysis and wrote the first draft. All authors discussed the results and contributed to the final manuscript.

### Conflict of Interest Disclosures

The authors report no conflicts of interest.

### Ethical Statement

Written informed consent was signed by the patient's parents and the research procedure was approved by the ethics committee of Semnan University of Medical Sciences (IR.SEMUMS.REC.1398.29).

### Acknowledgments

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